

#### STATE OF WEST VIRGINIA DEPARTMENT OF HEALTH AND HUMAN RESOURCES

Earl Ray Tomblin Governor

#### ENVIRONMENTAL PROGRAMS

Rocco S. Fucillo Cabinet Secretary

**February 8, 2013** 

To:

Accrediting Authorities

(EPA Region III and FDA-LPET)

From: Thomas L. Ong, HHR Program Manager

Associate Director of Environmental Programs

RE: Personnel Changes

This letter is to officially inform you of the following personnel changes within the Environmental Microbiology (Drinking Water and Grade A Dairy) Laboratory:

- > Effective January 16, 2013, Microbiologist Christopher B. Smith, Laboratory Certification Officer (Drinking Water Microbiology Program), terminated his employment with the West Virginia Department of Health and Human Resources.
- > Effective February 1, I was re-allocated to the position HHR Program Manager II, Associate Director of Environmental Programs, replacing Charlotte Billingsley who had been serving in the position part-time. I plan to maintain my status as a Laboratory Evaluation Officer in the Grade A Dairy Program.

If there are any questions, please do not hesitate to contact me.

BUREAU FOR PUBLIC HEALTH OFFICE OF LABORATORY SERVICES 167 11th Avenue South Charleston, West Virginia 25303-1134

Phone: (304) 558-3530

FAX: (304) 558-2006

DO

## On-Site Laboratory Evaluation **Update** Report (SDWA)

Date of Report: December 12, 2012

Microbiology -

Environmental Microbiology Section
Office of Laboratory Services
Bureau for Public Health
West Virginia Department of Health and Human Services
167 11<sup>th</sup> Avenue
South Charleston, WV 25303

Date of Assessment: September 18-19, 2012

Assessed by

David E. Russell

Environmental Science Center
Office of Analytical Services and Quality Assurance
U.S. Environmental Protection Agency, Region III
701 Mapes Road
Fort Meade, Maryland 20755-5350

#### A. Summary:

The corrective action plan dated November 16, 2012, and documentation indicating implementation, have been reviewed. These documents were produced in response to the EPA report resulting from the September 18-19, 2012, on-site assessment of the Environmental Microbiology Laboratory of the West Virginia Office of Laboratory Services. Based upon the review no changes in the certification status of methods from that listed in the initial on-site report are being recommended. However, although four outdated SOPs have been updated in response to the findings, the corrective plan indicates that the revisions of the SOPs for Multitube Fermentation, Heterotrophic Plate Count and Quality Control –Water Program are still in progress. These SOP updates were originally requested by December 19, 2012. Given the number of documents and the holiday season, the fully certified status listed in this update report will be extended until January 19, 2012 in anticipation of receipt of the additional SOPs.

In addition, certification for Modified Colitag (a new method requested by the Laboratory) is contingent upon submission of an acceptable SOP. Furthermore, it is noted here for the record, that the Laboratory no longer seeks certification for Membrane Filtration using SM9222B and SM9221E (total and fecal coliforms).

The findings for the microbiology quality system identified during the on-site were included in the on-site report for inorganic chemistry and are addressed in a separate update report.

The assessor appreciates the Laboratory's positive responses to the EPA recommendations in the on-site report.

#### B. Certification Status (Recommended by the Assessor):

Contingent upon the submission of acceptable SOPs for the methods noted above, the Assessor recommends the following SDWA certification status for each of the methods listed below.

Analyte	On-Site Method	On-Site Certification Status
Total Coliforms (Presence/Absence)	SM 9223 Colilert	Certified
Total Coliforms (Presence/Absence)	SM 9221B	Certified
Total Coliforms (Presence/Absence)	Modified Colitag	Certified
Fecal Coliforms (Presence/Absence)	- SM 9221B,E	Certified

Analyté	On-Site Method	On-Site Certification Status
Escherichia coli (E. coli) (Presence/Absence)	SM 9223 Colilert	Certified
Escherichia coli (E. coli) (Presence/Absence)	Modified Colitag	Certified
Escherichia coli (E. coli) (Enumeration)	SM 9223 Colilert QT	Certified
Heterotrophic Plate Count (HPC)	SM 9215B	Certified

C. Assessor:

12/12/12

David E. Russell Microbiological Assessor

Jon .

## **On-Site Laboratory Evaluation Report (SDWA)**

Date of Report: October 2, 2012

### Microbiology -

Environmental Microbiology Section
Office of Laboratory Services
Bureau for Public Health
West Virginia Department of Health and Human Services
167 11<sup>th</sup> Avenue
South Charleston, WV 25303

Date of Assessment: September 18-19, 2012

Assessed by

David E. Russell

Environmental Science Center
Office of Analytical Services and Quality Assurance
U.S. Environmental Protection Agency, Region III
701 Mapes Road
Fort Meade, Maryland 20755-5350

#### A. Introduction:

On September 18-19, 2012, an evaluation of the Environmental Microbiology Laboratory of the West Virginia Office of Laboratory Services, located in Charleston, was conducted to determine the capability of the Laboratory to perform its mission as it relates to the Safe Drinking Water Act. The Laboratory was last evaluated in September, 2009.

The Environmental Microbiology Laboratory (hereafter, the Laboratory) is currently analyzing water samples for total coliforms, fecal coliforms, *E. coli*, and heterotrophic bacteria using the methods shown below. It wishes to maintain certification for these methods.

Rule	Parameter	Method	
	Total Calif	SM 9221B	
	Total Coliforms	SM 9223 Colilert	
Total Coliform Rule	Fecal Coliforms	SM 9221B,E	
	E. coli	SM 9223 Colilert	
Long-Term 2 Enhanced Surface Water Treatment Rule	E. coli	SM 9223 Colilert QT*	
Not Applicable	Heterotrophic Bacteria	SM 9215B*	

<sup>\*</sup>quantitative result (all other methods shown produce a presence/absence result)

In addition the Laboratory seeks to acquire certification for the method known as Modified Colitag, a method similar to Colilert, but less expensive. Due to a lack of requests for analysis, the Laboratory has discontinued use of the membrane filtration method (SM9222B with SM9221E) for which it had been previously certified.

The assessment was conducted by Dave Russell, Microbiology Certification Officer, from EPA Region III, Office of Analytical Services and Quality Assurance, Environmental Science Center, 701 Mapes Road, Fort Meade, Maryland 20755-5350. In addition, Wanda Johnson, Drinking Water Program Manager, representing the Region III EPA Water Protection Division,

1650 Arch Street, Philadelphia, PA 19103-2029, participated via teleconference.

#### B. Personnel:

The following personnel currently analyze drinking water or source water for total coliforms, fecal coliforms, *E.coli*, or heterotrophic bacteria (heterotrophic plate count).

Tom Ong Microbiologist Supervisor
Mike Flesher Microbiologist III
Tracey Goodson Microbiologist III
Carole Moore Microbiologist II
Christopher Smith Microbiologist I

The assessor wishes to thank these individuals for their cooperation, willing assistance, and professionalism during the on-site evaluation. All are well-qualified experienced analysts.

#### C. Assessment and Data Audit Procedures:

The equipment, analytical procedures, and quality control practices employed in the bacteriological analyses of drinking water by the Laboratory were assessed for compliance with the requirements of the following.

- Manual for the Certification of Laboratories Analyzing Drinking Water, Criteria and Procedures Quality Assurance, Chapter V - Critical Elements for Microbiology, 5<sup>th</sup> Edition, January 2005, EPA 815-R-05-004 (CLADW)
- Supplement 1 to the Fifth Edition of the Manual for the Certification of Laboratories Analyzing Drinking Water, June 2008, EPA 815-F-08-006
- 40 CFR Part 141-143 SDWA requirements
- referenced analytical methods cited above in Section A
- technical SOPs
- Region III "musts" for Laboratory Quality Assurance Plans from Chapter III, CLADW (per the Region III Presurvey packages--chemistry and microbiology). (This change for continuous improvement was adopted by the Regional State Laboratory Directors and the EPA's Drinking Water Program since the last on-site assessment of the WV Office of Laboratory Services)

Key documents and data reviewed included the following.

- demonstration of capability performance results and supporting data,
- Proficiency Testing (PT) results and supporting data,
- EPA's last on-site inspection (9/22-23/09, report dated 10/15/09),
- Water Bacteriological Reports and supporting data for SDWA compliance samples for

May 2011 and May 2012,

• the Laboratory's 2012 certificate issued by Region III (effective January 1, 2012 until December 31, 2012).

The Assessor also observed the sample receipt and log-in procedure, as well as, the analysis of samples.

A Data Audit focused on Water Bacteriological Reports from May, 2011 and May, 2012. Records were reviewed that traced results from sample log-in to the final analytical results including related QC data. This provided a verification of completeness of documentation and the adequacy of the record keeping system.

"Findings" (see Section E) comprise procedures or practices that are not in compliance with the Manual for the Certification of Laboratories Analyzing Drinking Water, Criteria and Procedures, Quality Assurance, Chapter V - Critical Elements for Microbiology, 5<sup>th</sup> Edition, January 2005, EPA 815-R-05-004 (CLADW), other documents cited above, or that, in the opinion of the Assessor, jeopardize the generation of valid data.

#### D. Proficiency Testing (PT):

The Laboratory results for annual Proficiency Testing (PT) Studies were reviewed and are listed below. The Laboratory seeks certification for these methods.

Method	Analyte	Year / PT Study	Results
SM9223 Colilert	Total Coliforms and E.coli	2010 / WS163	Acceptable
SM9223 Colilert	Total Coliforms and E.coli	2011 / WS174	Acceptable
SM9223 Colilert	Total Coliforms and E.coli	2012 / WS186	Acceptable
SM9223 Colilert QT	E. coli	2010 / WS162	Acceptable
SM9223 Colilert QT	E. coli	2011 / WS174	Acceptable
SM9223 Colilert QT	E. coli	2012 / WS186	Acceptable

Method	Analyte	Year / PT Study	Results
Multiple-Tube Fermentation SM9221B,E	Total and Fecal Coliforms	2010 / WS162	Acceptable
Multiple-Tube Fermentation SM9221B,E	Total and Fecal Coliforms	2011 / WS175	Acceptable
Multiple-Tube Fermentation SM9221B,E	Total and Fecal Coliforms	2012 / WS187	Acceptable
Heterotrophic Plate Count SM9215B (Pour Plate)	Heterotrophic Bacteria	2010 / WS162	Acceptable
Heterotrophic Plate Count SM9215B (Pour Plate)	Heterotrophic Bacteria	2011 / WS174	Acceptable
Heterotrophic Plate Count SM9215B (Pour Plate)	Heterotrophic Bacteria	2012 / WS186	Acceptable
Modified Colitag	Total Coliforms and E.coli	2011 / WS180	Acceptable
Modified Colitag	Total Coliforms and E.coli	2012 / WS188	Acceptable

The Laboratory is to be congratulated on the excellent record of PT sample analysis established over the past three years.

#### E. Findings:

As noted above, Findings comprise procedures or practices that are not in compliance with the Manual for the Certification of Laboratories Analyzing Drinking Water, Criteria and Procedures, Quality Assurance, Chapter V - Critical Elements for Microbiology, 5<sup>th</sup> Edition, January 2005, EPA 815-R-05-004 (CLADW), other documents cited above (Section C), or that, in the opinion of the Assessor, jeopardize the generation of valid data.

1. Paragraph 8.4 (Chapter V, CLADW) lists the essential items to be captured in all analytical records. These include the result of the analysis. Currently, when performing presence/absence drinking water methods, the Laboratory only records on the bench sheet a positive (present) result for total coliforms. Fields left blank indicate negative (absent) results for total coliforms. Although it is understood within the Laboratory what the blank field indicates, this practice does not conform with paragraph 8.4 or with general conventional practice in microbiology. A result must be recorded for every test. SOPs need

to be updated to reflect this revised procedure. (It should be noted that both positive and negative results are recorded on the bench sheet for E. coli.)

- 2. Paragraph 11.3 (Chapter III, CLADW), as revised by Region III, states requirements for the management of laboratory SOPs. Although the Laboratory has in place a recently updated and signed SOP for Colilert (Presence/Absence) (Rev. 2.0 08/2012), SOPs for other methods are substantially out of date having not been revised or updated for several years. These include Colilert Quanti-Tray (2006), Multiple Tube Fermentation (2000), and Heterotrophic Plate Count (1998). Updated versions of these SOPs with signature pages need to be prepared.
- 3. Findings concerning aspects of the drinking water <u>microbiology quality system</u> reported in the Inorganic Chemistry and Laboratory Quality System Report (2012) are hereby referenced and incorporated into this report.

#### F. Recommendations:

The following remarks are offered as suggestions to help improve the quality and integrity of the data the Laboratory generates.

## It is requested that the laboratory provide feedback ("pros" and "cons") on the following suggestions offered in the spirit of continuous improvement:

- 1. Both paragraph 6.3.1 and the Federal Register (40 CFR 141.21(f)(3) footnote 2), in regard to the collection of drinking water samples from distribution systems, state, "Systems are encouraged but not required to hold samples below 10°C during transit." Accordingly, it is recommended that distribution system samples be held below 10°C during transit and that this condition be documented through the use of a temperature blank, the temperature of which would be determined upon arrival at the Laboratory and recorded.
- 2. According to paragraph 3.15.4, "sufficient sodium thiosulfate must be added to a sample bottle before sterilization to neutralize any residual chlorine in the water sample." The Laboratory currently uses sample bottles containing sodium thiosulfate in powder form. The Laboratory should consider ways to confirm that the sodium thiosulfate contained in sample bottles is "sufficient" to neutralize the residual chlorine present in the water sample. One suggestion is to routinely check (using a check box on the Water Bacteriological Report) that the residual chlorine recorded on the report form by the sample collector is a residual concentration that can be neutralized by the amount of sodium thiosulfate known to be in the sample bottle. According to IDEXX the pre-sterilized containers with sodium thiosulfate contain 10-35mg of sodium thiosulfate which will neutralize a minimum of 10 ppm residual chlorine. Laboratory staff could simply check the report form to be sure the residual chlorine recorded is less than or equal to 10 ppm and then check a box on the form documenting that

this procedure had been completed. Alternatively, it is suggested the Laboratory use commercially available potassium iodide strips to test a small aliquot of the sample, transferred to a watch glass, for the presence of residual chlorine. If present the strip will change color indicating that the sodium thiosulfate had not been sufficient to neutralize all the residual chlorine in the water sample. If the sodium thiosulfate had been sufficient, no residual chlorine would remain and the color change would not occur. Whatever means of performing this check is decided upon, it should be described in method SOPs or in the Laboratory's Quality Assurance Manual.

- 3. The Laboratory's Water Bacteriological Report (WBR) form captures a substantial amount of essential information, and the Laboratory is hereby commended for steadily improving it through a number of revisions. When next revised, or even prior to the next revision (because space on the form is currently available directly under "Laboratory Results"), it is recommended that the Laboratory include the <u>date and time results are read</u> on the form. This information is currently documented on the laboratory bench sheet, however, it is the only critical piece of information missing from the WBR. All other key dates and times are currently recorded on the WBR. Addition of the <u>date and time results are read</u> would make the WBR <u>a complete record of sample collection</u>, receipt and analysis. Currently neither the bench sheet or the WBR captures everything.
- 4. A further improvement of the Water Bacteriological Report would be to include the <u>units</u> of the chlorine residual recorded by the sample collector. This should be added to the training provided sample collectors with an explanation of why determining and recording the chlorine residual is so important (so that the lab can evaluate the sufficiency of the amount of sodium thiosulfate added to the sample bottles prior to sample collection).
- 5. Although a few years ago the Laboratory significantly improved its record of thermometer calibration by devoting one page of the log book to each thermometer--allowing anyone to easily review the history of calibration of any one thermometer--the current record system has moved a bit away from that simple system. Currently, one page is devoted to each thermometer when it is in a <u>particular location</u>. So a single thermometer may have several pages, each a different location. It is recommended each thermometer have one page devoted to its calibration and that the location of the thermometer simply be a field on the line documenting a thermometers annual calibration.

#### G. Modified Colitag:

The Laboratory is also seeking certification for Modified Colitag, an EPA-approved presence/absence drinking water method. As shown in Section D, the Laboratory has successfully analyzed PT samples with this method for two consecutive years (2011 and 2012). In order to obtain certification, the Laboratory needs to submit an acceptable SOP (with signature page) and pages from logs documenting QC of media.

#### **H.** Conclusions:

The Laboratory's management and staff are to be commended for their dedication to maintaining high standards in microbiological analysis and remaining committed to continual improvement. In particular, the Laboratory is to be commended for the routine practice of rejecting samples (without analysis) when necessary for the reasons listed on the Water Bacteriological Report. The Laboratory is to be further commended for the documentation of QC using well-designed MS Excel spreadsheet logs containing embedded formulas for routine calculations and embedded automatic evaluations of QC data.

As shown in the table below, certification will be recommended for all methods requested, (except Modified Colitag as noted above) contingent upon receipt of an acceptable corrective action plan addressing the findings in Section E.

#### I. Corrective Actions:

The listed findings need be addressed in the corrective action summary prepared by the laboratory within 30 days of the receipt of the assessment report. To help speed the review and response to the summary, please fill in the response after each listed finding in this report and reference documentation of corrective action by number in the response). To the degree possible, the corrective action plan and supporting documentation of implementation should be provide in electronic format. Documentation of full implementation of all corrective actions is requested within 60 days of receipt of this report (90 days from the on-site assessment closing meeting).

#### J. Certification Status (Recommended by the Assessor):

Based upon the on-site assessment and upon submission of acceptable corrective actions to address the findings listed in Section E, the Assessor recommends the following SDWA certification status for each of the following methods.

Analyte	On-Site Method	On-Site Certification Status
Total Coliforms (Presence/Absence)	SM 9223 Colilert	Certified
Total Coliforms (Presence/Absence)	SM 9221B	Certified
Fecal Coliforms (Presence/Absence)	SM 9221B,E	Certified

Analyte	On-Site Method	On-Site Certification Status
Escherichia coli (E. coli) (Presence/Absence)	SM 9223 Colilert	Certified
Escherichia coli (E. coli) (Enumeration)	SM 9223 Colilert QT	Certified
Heterotrophic Plate Count (HPC)	SM 9215B	Certified

K. Assessor:

Dayid E. Russell Microbiological Assessor





## STATE OF WEST VIRGINIA DEPARTMENT OF HEALTH AND HUMAN RESOURCES

**ENVIRONMENTAL MICROBIOLOGY** 

Rocco S. Fucillo Cabinet Secretary

Earl Ray Tomblin Governor

November 16, 2012

David E. Russell, Ph.D.
U.S. Environmental Protection Agency, Region III
Office of Analytical Services and Quality Assurance
701 Mapes Road
Fort Meade, MD 20755-5350

Dear Dr. Russell,

The following is a list of corrective actions along with supporting documentation that have taken place since the September 18-19, 2012 triennial on-site evaluation at the Office of Laboratory Service's Drinking Water Laboratory:

1. Paragraph 8.4 (Chapter V, CLADW) lists the essential items to be captured in all analytical records. These include the result of the analysis. Currently, when performing presence/absence drinking water methods, the Laboratory only records on the bench sheet a positive (present) result for total coliforms. Fields left blank indicate negative (absent) results for total coliforms. Although it is understood within the Laboratory what the blank field indicates, this practice does not conform with paragraph 8.4 or with general conventional practice in microbiology. A result must be recorded for every test. SOPs need to be updated to reflect this revised procedure. (It should be noted that both positive and negative results are recorded on the bench sheet for E. coli.)

**Response:** Absents are now recorded in the bench sheets. The SOP's reflect this. See the Attached SOPs: SOPEMW-002-R3.0, "Standard Methods 9223, Enzyme Substrate, Colilert/Colilert18 100 mL" and SOPEMW-003-R2.0, "Standard Methods 9223 QT, Enzyme Substrate, Colilert/Colilert18 Quanti Tray".

2. Paragraph 11.3 (Chapter III, CLADW), as revised by Region III, states requirements for the management of laboratory SOPs. Although the Laboratory has in place a recently updated and signed SOP for Colilert (Presence/Absence) (Rev. 2.0 – 08/2012), SOPs for other methods are substantially out of date having not been revised or updated for several years. These include Colilert Quanti-Tray (2006), Multiple Tube Fermentation (2000), and Heterotrophic Plate Count (1998). Updated versions of these SOPs with signature pages need to be prepared.

**Response:** All SOPs are undergoing a major revision. The following SOPs have been completed, signed and included as attachments to this response:

BUREAU FOR PUBLIC HEALTH OFFICE OF LABORATORY SERVICES . 167 11<sup>th</sup> Avenue South Charleston, West Virginia 25303-1134

Phone: (304) 558-3530

FAX: (304) 558-2006

- > DCN: SOPEMW-001-R1.0, "Sample Handling: Collection, Accessioning and Reporting"
- DCN: SOPEMW-002-R3.0, "Standard Methods 9223, Enzyme Substrate, Colilert/Colilert18 100 mL"
- ➤ DCN: SOPEMW-003-R2.0, "Standard Methods 9223 QT, Enzyme Substrate, Colilert/Colilert18 Quanti Tray"
- > DCN: SOPEMS-007-R1.0, "Ethics and Data Integrity"

DCN: SOPEMW-004-R2.0, "Multi Tube Fermentation 100 mL & 10 x 10 mL"; DCN: SOPEMW-005-R1.0, "Heterotrophic Plate Count" and DCN: SOPEMW-006-R1.0, "Quality Control – Water Program" are currently in the works and will be submitted as they are completed and signed.

3. Findings concerning aspects of the drinking water microbiology quality system reported in the Inorganic Chemistry and Laboratory Quality System Report (2012) are hereby referenced and incorporated into this report.

**Response:** The entire Quality System of the Drinking Water Microbiology Program is being "over-hauled". The findings listed in the *Inorganic Chemistry and Laboratory Quality System Report* are addressed in a separate correspondence.

#### F. Recommendations:

The following remarks are offered as suggestions to help improve the quality and integrity of the data the Laboratory generates.

It is requested that the laboratory provide feedback ("pros" and "cons") on the following suggestions offered in the spirit of continuous improvement:

1. Both paragraph 6.3.1 and the Federal Register (40 CFR 141.21(f)(3) footnote 2), in regard to the collection of drinking water samples from distribution systems, state, "Systems are encouraged but not required to hold samples below 10°C during transit." Accordingly, it is recommended that distribution system samples be held below 10°C during transit and that this condition be documented through the use of a temperature blank, the temperature of which would be determined upon arrival at the Laboratory and recorded.

**Response:** This would require the purchase of special shipping containers and freeze packs to accommodate hundreds of samples. It would also dramatically increase operating costs for both the laboratory and public water supplies by increasing the postage for the added size and weight of the new containers. At this time, it is not fiscally practical to accomplish this.

2. According to paragraph 3.15.4, "sufficient sodium thiosulfate must be added to a sample bottle before sterilization to neutralize any residual chlorine in the water sample." The Laboratory currently uses sample bottles containing sodium thiosulfate in powder form. The Laboratory should consider ways to confirm that the sodium thiosulfate contained in sample bottles is "sufficient" to neutralize the residual chlorine present in the water sample. One suggestion is to routinely check (using a check box on the Water Bacteriological Report) that the

residual chlorine recorded on the report form by the sample collector is a residual concentration that can be neutralized by the amount of sodium thiosulfate known to be in the sample bottle. According to IDEXX the pre-sterilized containers with sodium thiosulfate contain 10-35mg of sodium thiosulfate which will neutralize a minimum of 10 ppm residual chlorine. Laboratory staff could simply check the report form to be sure the residual chlorine recorded is less than or equal to 10 ppm and then check a box on the form documenting that this procedure had been completed. Alternatively, it is suggested the Laboratory use commercially available potassium iodide strips to test a small aliquot of the sample, transferred to a watch glass, for the presence of residual chlorine. If present the strip will change color indicating that the sodium thiosulfate had not been sufficient to neutralize all the residual chlorine in the water sample. If the sodium thiosulfate had been sufficient, no residual chlorine would remain and the color change would not occur. Whatever means of performing this check is decided upon, it should be described in method SOPs or in the Laboratory's Quality Assurance Manual.

**Response:** Although this is a recommendation under the microbiology report, it appears to be a requirement under the Quality Systems Report, Item 1.:

- "1. The procedures used for <u>sample receiving</u> need to be documented for environmental chemistry and microbiology [Region III "musts" for CLADW, III-5, 11.1, 11.4 &11.5]. This needs to include:
  - recording information in the Sample Log-in book and the Star LIMS,
  - the numbering system for samples including the designation of "regulated" and "COC", etc.;
  - · checking and recording of necessary preservation checks; and
  - who is responsible (position) for these tasks.

We have implemented using Potassium Iodide Strips and documenting the results. The procedure is outlined in each method SOP. See Attached.

3. The Laboratory's Water Bacteriological Report (WBR) form captures a substantial amount of essential information, and the Laboratory is hereby commended for steadily improving it through a number of revisions. When next revised, or even prior to the next revision (because space on the form is currently available directly under "Laboratory Results"), it is recommended that the Laboratory include the date and time results are read on the form. This information is currently documented on the laboratory bench sheet, however, it is the only critical piece of information missing from the WBR. All other key dates and times are currently recorded on the WBR. Addition of the date and time results are read would make the WBR a complete record of sample collection, receipt and analysis. Currently neither the bench sheet or the WBR captures everything.

**Response:** Water Bacteriological Report Forms are currently being printed. This change was not included on the revision that is currently being printed; however, it may be considered for future revisions.

4. A further improvement of the Water Bacteriological Report would be to include the units of the chlorine residual recorded by the sample collector. This should be added to the training provided sample collectors with an explanation of why determining and recording the chlorine

EPA On-Site Evaluation Response November 16, 2012 Page Page **4** of **4** 

residual is so important (so that the lab can evaluate the sufficiency of the amount of sodium thiosulfate added to the sample bottles prior to sample collection).

**Response:** Water Bacteriological Report Forms are currently being printed. This change was included on the revision that is currently being printed. A sample of the changes being made is demonstrated in Appendix B of DCN: SOPEMW-001-R1.0, "Sample Handling: Collection, Accessioning and Reporting"

5. Although a few years ago the Laboratory significantly improved its record of thermometer calibration by devoting one page of the log book to each thermometer--allowing anyone to easily review the history of calibration of any one thermometer--the current record system has moved a bit away from that simple system. Currently, one page is devoted to each thermometer when it is in a particular location. So a single thermometer may have several pages, each a different location. It is recommended each thermometer have one page devoted to its calibration and that the location of the thermometer simply be a field on the line documenting a thermometers annual calibration.

**Response:** This will be incorporated in the new Water Room Quality Control Book. The plan is to further separate the common items from the Water Program and the Dairy Program such as Balances, Pippettes, Thermometers, etc.

The staff, administration and I would like to thank you for the positive evaluation report. All staff, myself included, learned from this experience and are constantly seeking to improve and streamline procedures and techniques. If you need additional information, please don't hesitate to ask.

Sincerely,

Thomas L. Ong, HHR Program Manager I Chief – Laboratory Certification Officer (Micro)

Date: February 27, 2012

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# Attachment # 5 B Pre-Survey Questionnaire/Checklist & Microbiology:

Laboratory:	West Virginia Department of Health and Human Resources
	Bureau For Public Health
	Office of Laboratory Services
Mailing Add	lress (mailing address of owner if different):
Street: 167	- 11 <sup>th</sup> Avenue
City, State, 2	Zip code: South Charleston, WV 25303
Audit Locati	on (if different):
Telephone:	304-558-3530, ext 2710
Fax: 304-55	8-2006
E-mail: Ton	n.L.Ong@wv.gov
Other:	
Audit Organ	ization: United States Environmental Protection Agency
Auditors/Sig	natures:
Audit Date(s	): Microbiology: September 18-19, 2012 Program Review: September 20-21, 2012

Note: Please complete and submit this questionnaire and additional requested material in electronic format.

"I"

- Used by FR DA Assessor, Done Russell,

- to indicate confirmation of WV's response

- Presurey question.

Date: February 27, 2012

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#### II. Laboratory Administration

(Please copy if more space is required)

**Laboratory Administration** 

	TRAIN	ING_		YEARS OF E		LIST SDWA METHODS CURRENTLY	
NAME	DEGREE (Circle One)	MAJOR	POSITION	PRESENT JOB	PREVIOUS JOB	PERFORMING	
Andrea Labik	Sc.D MS BS/BA Assoc. HS	Public Health Microbiology	Director	13 Years	29 Years		
Thomas L. Ong	Ph.D MS BS/BA Assoc. HS		Quality Assurance Officer				
· .	Ph.D MS BS/BA Assoc. HS		Laboratory Contact Person (if different from the Director)			7	
Thomas L. Ong	Ph.D MS BS/BA Assoc. HS	Biology	Section Supervisor: <u>Microbiology</u>	16 Years	7 Years	SM 9221 B/E SM 9223 B (Colilert) SM 9223 B OT SM 9215 B	
	Ph.D MS BS/BA Assoc. HS	·	Section Supervisor:				
	Ph.D MS BS/BA Assoc. HS		Section Supervisor:				
	Ph.D MS BS/BA Assoc. HS		Section Supervisor:				

**Laboratory Personnel** 

Date: February 27, 2012

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6

TRAINING YEARS OF EXPERIENCE LIST SOWA METHODS CURRENTLY PERFORMING NAME DEGREE **POSITION** PRESENT JOB **PREVIOUS** MAJOR (Circle One) JOB SM 9221 B/E/ Ph. D MS Michael Flesher SM 9223 B Public Microbiologist III, Laboratory 9 Years 7 Years BS/BA Health/ Certification Officer SM 9223 B QT SOUND ( Assoc Biology SM 9215 B Ph. D SM 9221 B/E **Tracy Goodson** Biology/ Microbiologist III, Laboratory 6 Years 4 Years SM 9223 B BS/BA/RN **Certification Officer** SM 9223 B QT Nursing Assoc. HS AWAS SM 9215 B Ph. D SM 9221 B/E L.E.O. MS Carole Moore Biology Microbiologist II 3 Years 3 Years SM 9223 B BS/BA SM 9223 B QT MILL C.O. Assoc. SM 9215 B HS Ph. D MS SM 9221 B/E **Christopher Smith** Bd. Of SM 9223 B Microbiologist I 1 Year 3 Years BS/BA Reagents/ SM 9223 B QT Assoc. AWGR Env. Tech SM 9215 B HS Ph. D MS BS/BA Assoc. HS Ph. D MS BS/BA Assoc. HS Ph. D BS/BA Assoc.

Date: February 27, 2012

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## Type in "YES" in the "Certification Requested" Column If Your Laboratory is Requesting SDWA Certification:

**Note:** The areas in Red are required for primacy per CFR and either the PSL must be certified or the State's Drinking Water Program have commercial laboratories holding current NELAP accreditations for these analytical areas. "Or" in the method citation column indicates an alternative. A "," indicates both citations are required and a "\(\Rightarrow\)" indicates transfer to new media.

Please double check your "Yes" entries ("Certification Requested").

Approved Methods	Section in Cert. Manual	Media	Method Citation?	Certification Requested? (Yes/No)
Total Coliforms (Prese	nce/Absenc	ce), Total Coliform Rule (TC	R)	
Fermentation broth method	5.2.2.	,LTB⇒BGLB Broth	SM 9221B	Yes
*	5.2.3	P-A Broth → BGLB Broth	SM 9221D	
Ç.	5.3.2	Colilert®, Colilert 18®	SM 9223 Colilert	Yes
	5.3.2	Colisure®	SM 9223 Colisure	
Enzyme substrate	5.3.2	Readycult® or Fluorocult LMX®	Readycult or Fluorocult LMX	
	5.3.2	E*Colite®	E*Colite	
<del>مبيم</del> الماريخ الراريخ	5.3.2	Modified Colitag®	Modified Colitag	Yes
	5.4.2	M-Endo or LES-Endo → LTB, BGLB Broth	SM 9222B or SM 9222 B,C	Dropping 2
Membrane filter method	5.4.2	MI Medium	EPA 1604	,
ears a	5.4.2	m-ColiBlue 24®	m-ColiBlue 24	
	5.4.2	Chromocult® -	Chromocult	
	5.4.2	Coliscan®	Coliscan '	
Fecal Colifornis (Prese	nce/Absenc	e), Total Coliform Rule (TC	R) /E+E¢	
Fermentation broth	5.2.4	LTB or P/A broth = ÉC broth	SM:9221B,E or SM:9221D,E	Yes
	5.2.4	A-1 broth	SM 9221E (A1)	
Membrane filter method	5.2.4	M-Endo medium ⇒ EC broth	SM 9222B, SM9221E	
	5.4.2	mFC	SM 9222D	

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Approved Methods	Section in Certa Manual	Media	Method Citation <sup>1</sup>	Certification Requested? (Yes/No)	
これのは、 はない。 はないないできた。 となるない。 はない、 とないます。 はないないできた。 とないます。 はないないできた。 はないないないできた。 はないないないできた。 はないないないないないないないないないないないないないないないないないないない	ence/Absen	ce), Total Coliform Rule (TO	R)		
سندن سنسب ساعد عالم	5.3.2	Colilert® , Colilert-18®	SM 9223 Colilert	Yes	
	5.3.2	Colisure®	SM 9223 Colisure		
Enzyme substrate method	5.3.2	E-Colite®	E-Colite		
	5.3.2	Readycult® or Fluorocult LMX®	Readycult or Fluorocult LMX		
· .	5.3.3	LTB, P/A broth, or M-Endo ⇒ EC-MUG	SM 9221B,F or SM 9221D,F or SM 9222B, SM 9221F or SM 9222B,G		·
	5.3.2	Modified Colitag®	Modified Colitag	Yes	9
Membrane filter	5.4.2	MI Medium	EPA 1604		
memod	5.4.2	m-ColiBlue24®	m-ColiBlue24		·
	5.4.2	Chromocult®	Chromocult		
	5.42	Coliscan®	Coliscan		
·	5.4.3	M-Endo/LES Endo → NA-MU	SM 9222B,G		
Escherichia coli (Enur	neration), L	ong Term 2 Enhanced Surfa	ce Water Treatment Rule (LT2		LT2 <sup>4</sup>
Enzyme Substrate	LT2* {	Colilert <sup>© 4</sup> , Colilert 18 <sup>© 4</sup>	SM 9223 Colilert QT	Yes	
Method	LT2+	M-Endo/LES-Endo → NA-MUG <sup>2,4</sup>	SM 9222B,G		
Membrane filter method	LT2 <sup>4</sup>	mFC → NA-MUG <sup>4</sup>	SM 9222D,G		
	LT2 <sup>4</sup>	mTEC ⁴	EPA 1103.1 or SM9213D		l
	LT2 <sup>4</sup>	Modified mTEC <sup>4</sup>	EPA 1603		
	LT2 <sup>4</sup>	MI Medium <sup>4</sup>	EPA 1604		

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- Approved Methods	Section in Cert. Manual	Media	Method Citation.	Certification Requested? (Yes/No)	
fis is	LT2 <sup>4</sup>	m – ColiBlue24® <sup>2,4</sup>	m – ColiBlue24		t.
	LT2 <sup>4</sup>	m – ColiBlue24® <sup>2,4</sup>	m – ColiBlue24		
Heterotrophic Bacteria	ı (Enumera	tion) Surface Water Treatme	nt Rule (SWTR) & TCR		5.5
Pour plate method	5.5	SimPlate@ Pour	SimPlate SM 9215B	Yes	
Multiple enzyme substrate	5.5	SimPlate Pour Contract Agriv RZA Sin plate	SM 9215B (R2A) or SM 9215C (R2A) or SM 9215D (R2A)	1 cla	·
Pour plate, spread plate, or membrane filter methods	5.5	R2A	SM 9215B (R2A) or SM 9215C (R2A) or SM 9215D (R2A)		
⊬Enterococci, (Presenc	e/Absence)	Ground Water Rule (GWR)	PT must:be+/- as 10 sample V	VS	5.3.4
Enzyme substrate	5.2.5	Azide Dextrose → BEAA → BHI	SM 9230B		
Fermentation broth method	5.4.4	mE ⇒ EIA	SM 9230C		
Membrane filter method	5.4.4	mEI	EPA 1600		

<sup>&</sup>lt;sup>1</sup> SM = Standard Methods for the Examination of Water and Wastewater, 18<sup>th</sup>, 19<sup>th</sup>, 20<sup>th</sup> and 21<sup>st</sup>. The on-line edition is not included in this listing.

<sup>&</sup>lt;sup>2</sup> If high levels of non-*E. coli* total coliforms interfere with the ability to accurately enumerate *E. coli* despite additional dilutions, an alternate method should be used (i.e., SM 9222D/9222G, SM 9213D/EPA Method 1103.1, EPA method 1603, SM 9221B.1/9221F, and SM 9223B).

<sup>&</sup>lt;sup>3</sup> Based on Ground water Rule. Water Supply (<u>WS</u>) PT study participation is required (WS as a special +/- set of ten).

<sup>&</sup>lt;sup>4</sup> To be performed in compliance with the *Microbial Laboratory Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule*, (EPA 815-R06-006) USEPA Office of Water, February, 2006 (http://www.epa.gov/ogwdw/disinfection/lt2/pdfs/guide\_lt2\_microbialguidancemanual.pdf). PT must be quantitative. Large water systems are required to monitor cryptosporidium for 24 months but may reduce cost by monitoring for E. coli enumeration for 12 of the 24 months. EPA OGWDW currently performs all certifications for cryptosporidium.

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Quality Assurance (Please reference each item to the section # & page number of the Laboratory Quality Assurance Manual/Plan (QAP/QM). If there is a separate Microbiology QAP/QM see a similar table in the Chemistry Presurvey form) Items in Yellow are "musts" in Region 3.

Quality Assurance Topic (CLADW, Chapter III Section 11)	Section & Page Number	Comment
Laboratory organization and responsibility (CLADW Section 11.1)		
Include a chart or table showing the laboratory organization and lines of responsibility, including QA managers;	13-14	
List the key individuals who are responsible for ensuring the production of valid measurements	5-5	
and the routine assessment of measurement systems for precision and accuracy (e.g., who is responsible for internal audits and reviews of the implementation of the plan and its requirements; quality system duties of QAO & Lab Managers)	10	
Sample custodian duties and log-in procedures	54-55	
Reference the job descriptions of the personnel and describe training to keep personnel	15-16	<del></del>
updated on regulations and methodology, and document that laboratory personnel have demonstrated proficiency for the methods they perform.	6-7	
Process used to identify clients' Data Quality Objectives (CLADW Section 11.2)		
A description of communications with SDWA customers (compliance monitoring).	57	College of the Principle of College, American Service of the Servi
OPs with dates of last revision. (CLADW Section 11.3)		
The laboratory should maintain SOPs that accurately reflect all phases of current laboratory activities	20	Barbara and Art Sala Progress of the Art Sala Sala Sala Sala Sala Sala Sala Sal
Keep a list of SOPs	20	
Ensure that current copies of SOPs are in the laboratory and in the QA Managers files;	20	······································
Ensure that SOPs are reviewed annually and revised as changes are made;	20	
1		

Date: February 27, 2012 Page 8 of 71 DCN: R3-QA801.4 The laboratory must maintain SOPs that accurately reflect all phases of current laboratory 20 activities; keep a list of SOPs; ensure that current copies of SOPs are in the laboratory and in the QA Managers files; ensure that SOPs are reviewed annually and revised as changes are made; ensure that SOPs have signature pages and revisions dated. (Section 11.3, p. III-4) Field sampling procedures (CLADW Section 11:4) Describe the process used to identify sample collectors, sampling procedures and locations, 41 required preservation, proper containers, correct sample container cleaning procedures, sample holding times from collection to analysis, and sample shipping and storage conditions Ensure that appropriate forms are legibly filled out in indelible ink or hard copies of electronic 41 data are available. See Chapters IV, V, and VI for specific items to be included Describe how samples are checked when they arrive for proper containers and temperature and 54-55 how samples are checked for proper preservation (e.g., pH, chlorine residual) before analysis Ensure that sampling protocol is written and available to samplers 54-55 Laboratory sample receipt and handling procedures (CLADW Section 11.5) Region III encourages a strong sample rejection policy as opposed to flagging sample results. Bound laboratory note books, if used, "must" be filled out in ink; entries dated and signed (A \*\*Will update this section and forward within a secure, password protected, electronic data base is acceptable); week. We received an update to our database program in June. The new SOP addressing the Store unprocessed and processed samples at the proper temperature, isolated from laboratory current work flow is 75% complete and will be contaminants, standards and highly contaminated samples and, sometimes, each other; holding times may not be exceeded forwarded within a week. Maintain integrity of all samples, (e.g., by tracking samples from receipt by laboratory through analysis to disposal); Require Chain-of-Custody procedures for samples likely to be the basis for an enforcement action or as evidence for litigation Specify criteria for rejection of samples which do not meet shipping, holding time and/or

preservation requirements and procedures for notification of sample originators

than two hours.

**6.3.3** For SWTR samples and coliphage samples, sample temperature upon receipt should be recorded. A sample that has a temperature upon receipt of >10C, whether iced or not, should be flagged unless the time since sample collection has been less

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**Holding time for microbiology** is (<u>must</u>) the time from collection until placement in the incubator. (Supplement #1, EPA 815-F-08-006, June 2008):

or whenever the thermometer has been exposed to temperature extremes.

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	Quality Assurance Topic	Page Number	Comment
L	strument calibration procedures (may reference SOP) (CLADW Section 11.6)		
•	Specify type of calibration used for each method and frequency of use		Equipment/Reagent SOP
٠	Describe calibration standards' source, age, storage, labeling		Not Addressed
•	<b>7.1.6 Traceability of Calibration:</b> Calibrations of all measurement devices <u>must</u> be traceable to national standards whenever applicable (Section 7.1.6, p. IV-4)	,	Not Addressed
	7.1.3 Balances and Weights:  a. Balance range "must" be appropriate for the application for which it is to be used. Drinking water chemistry laboratories "must" use balances that weigh to at least 0.0001 g. The balances "must" be calibrated at least annually with ASTM Type I, Class 1 or 2 weights. (ASTM, 1916 Race St., Philadelphia, PA 19103) Weights meeting ASTM Type I, Class 1 or 2 specifications "must" be recertified at least every five years or if there is reason to believe damage (corrosion, nicks) has occurred.		SOP – Balance Calibrations
•	b. Each day the mechanical or digital balance is used, a verification "must" be performed. The verification consists of a check of a reference mass at approximately the same nominal mass to be determined. Verifications should be done each weighing session unless it can be shown that fluctuations in the environment do not affect the calibration. The checks and their frequency should be as prescribed in the laboratory's QA Plan. A record of all checks should be kept and be available for inspection.	???	Chemistry Requirement
•	7.1.5 Temperature Measuring Devices Liquid bearing thermometers such as mercury or alcohol thermometers "must" be traceable to NIST calibration and verified at least annually and whenever the thermometer has been exposed to temperature extremes. The correction factor should be indicated on the thermometer and the date the thermometer was calibrated and the calibration factor should be kept as prescribed in the laboratory's QA documents and be available for inspection. The NIST thermometer "must" be recalibrated at least every five years	,	Equipment/Reagent SOP

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"must" be verified at lemperature range the iced (4°C) and frozen checking the tempera calibrated thermomet	ection device is used to measure the temperature of samples least every six months using a NIST certified thermometer ov at the IR thermometer will be used. This would include ambie (0 to -5C). Each day of use a single check of the IR should be ture of a bottle of water at the temperature of interest that coler. Agreement between the two "must" be within 0.5C, or the d. (Section 7.1.3 p. IV-4)	er the full ???? nt (20-30°C), e made by ntains a	Chemistry Requirement
Analytical procedure	s (may reference SOP) (CLADW Section 11.7)		
Cite complete method		20	·
Specify that the quality	ty control procedures required by the required methods need	to be followed	Specified in each method SOP
coliforms/100 mL, etc			
	ion process: method of conversion of raw data to mg/L, picoc	uries/L,	
	: <u>ion_process_(assuring</u> data meets the customer's data quality	y needs)	**Will update this section and forward wit week. We received an update to our datab
Describe data <u>validat</u>		y needs)	week. We received an update to our datab program in June. The new SOP addressing current work flow is 75% complete and wi
<ul><li>Describe data <u>validat</u></li><li>Describe reporting pr</li></ul>	ion process (assuring data meets the customer's data quality	y needs)	
<ul><li>Describe data <u>validat</u></li><li>Describe reporting pr</li></ul>	ion process (assuring data meets the customer's data quality ocedures, include procedures and format ation process (assuring data reported is what was obtained)	y needs)	week. We received an update to our datab program in June. The new SOP addressing current work flow is 75% complete and wi
<ul> <li>Describe data validat</li> <li>Describe reporting pr</li> <li>Describe data verification</li> <li>Describe procedure for</li> </ul>	ion process (assuring data meets the customer's data quality ocedures, include procedures and format ation process (assuring data reported is what was obtained)	y needs)	week. We received an update to our datab program in June. The new SOP addressing current work flow is 75% complete and wi
<ul> <li>Describe data validat</li> <li>Describe reporting pr</li> <li>Describe data verification</li> <li>Describe procedure for the quality system not</li> </ul>	ion process (assuring data meets the customer's data quality ocedures, include procedures and format ation process (assuring data reported is what was obtained) or data corrections or validation/verification of software and LIMS eeds to (must) describe the quality system for the Laboratory	Information	week. We received an update to our datab program in June. The new SOP addressing current work flow is 75% complete and wi
Describe data validat  Describe reporting pr  Describe data verificat  Describe procedure fruit of the data verificate  Describe procedure fruit of the data verificate  The quality system not management System Section 8.1, page IV-	ion process (assuring data meets the customer's data quality ocedures, include procedures and format ation process (assuring data reported is what was obtained) or data corrections or validation/verification of software and LIMS	Information LADW	week. We received an update to our datab program in June. The new SOP addressing current work flow is 75% complete and wi

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٠	2) Define the roles of other staff in monitoring the function and procedure (including			
	maintenance) of the LIMS. The latter should be independent of LIMS personnel, report directly			•
	to laboratory management. This needs to include periodic audits to ensure integrity.			
٠	Have documented procedures and practices to verify the accuracy of LIMS raw data			
	including the individuals responsible for entering data.			
٠	4) Have documented procedures for testing and quality assurance of hardware and software,			
	including procedures for tracking software versions and records of verifications	<u></u>		
٠	5) Have documented security procedures to protect LIMS data.			
			*	
٠	6) Have documented procedures for testing, inspecting and maintaining new LIMS hardware.			`
٠	7) Have documented procedures to assure the retention of laboratory records that comply with			
	regulations (CLADW specifies record retention of at least 5 years for lab records and 6 years for			
	inspection records). For example, the procedures for backing up the LIMS including who is			
	responsible, the frequency, procedures and where this is documented.			
			•	
٠	Have documented listing of facility requirements for the LIMS.			/

T	ype of quality control (QC) checks and the frequency of their use.(may reference SO	P) (CLAD	W Section 11.9)
٠	Laboratories performing analysis of drinking water under the Safe Drinking Water Act (SDWA)		
	are required to operate a formal Quality Control program. Laboratories "must" also have a	2 .	
	formal Quality Management system documented and in place. (Supplement #1, EPA:815-F-08-		
	006, June 2008		
٠	Positive & negative Controls		Each Method SOP
٠	Precision measurements performed on HPC		Not Addressed
٠	Field QC (field blanks, fields duplicates)		Each Method SOP
٠	13.1 Proficiency Testing (PT) Samples		,
	At least annually drinking water laboratories certified for microbiological contaminants must	99	<u>†</u>
	satisfactorily analyze a PT sample to maintain certification (40CFR		
	141.23(k)(3)(i),141.24(h)(17)(i)(A) and 141.89(a)(1)(i)). PT samples "must" be analyzed in the		
	same manner as routine samples. Laboratories must acquire the PT sample from a supplier		
i	acceptable to the appropriate certification authority.		·

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	The laboratory "must" be able to provide documentation to the certification authority that the person(s) analyzing any PT sample is a laboratory employee who routinely analyzes drinking water compliance samples. (Section 13.1, p. III-6)		
•	Initial demonstration of method capability/performance (e.g., successful QC/PT analyses)	95	
•	Qualitative identification/confirmation		Each Method SOP
•			Each Method SOP
	Acceptance limits for QC checks (e.g., use of control charts, limits from reference methods, etc.)		·
•	All QC information must be readily available to auditors. (Section 7.1.2, p. IV-4)	89	

Quality Assurance Topic	Page Number	Comment
List schedules of internal and external system and data quality audits and inter labor Section 11.10)	ratory comparisons (	may reference SOP) (CLADW
Internal reviews conducted of technical operations, frequency and corrective actions	10	
• Internal reviews done by management to assure the quality system is effective and appropriate.	81-86	
Preventive maintenance procedures and schedules (CLADW Section 11:11)  Describe location of instrument manuals and schedules and documentation of routine equipment maintenance	93	
Describe availability of instrument spare parts in the laboratory	93	
List any maintenance contracts in place	93	
Corrective action contingencies (CLADW Section 11.2)		
Describe corrective actions upon obtaining unacceptable results from analysis of PT samples	95.99	

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and from internal QC checks including "root cause analysis"	
Name persons responsible for the various corrective actions	95, 99
Describe how corrective actions taken are documented (implementation)	95, 99
Record keeping procedures (CLADW Section 11.13)	
Describe procedures and documentation of those procedures;	89
List length of storage, media type (electronic or hard copy)	89
Describe security policy of electronic databases	91
All electronic data should have software support so it may be regenerated	91
<ul> <li>QA Plan <u>must</u> describe policies and procedures to keep thorough and accurate records, records integrity and retention/storage. Compliance monitoring data <u>must</u> be associated with thorough and accurate records. (8.1)</li> </ul>	
<ul> <li>If samples are expected to become part of a legal action, chain of custody procedures <u>must</u> be used (See Appendix A). (Section 8.1, p. IV-8)</li> </ul>	be 75
Laboratory <u>must</u> maintain easily accessible records for 5 years. (Section 8.2, p. IV-8)	89 .
<ul> <li>Adequate information <u>must</u> be available to allow the auditor to reconstruct the final results for compliance samples and PT samples. (Section 8.5, p. IV-8)</li> </ul>	89
• Data Integrity/Ethics (CLADW Supplement 1, EPA 815-F-08-006, June 2008)	
• Ethics policy	**Will forward this section prior to on-site.  SOP is under review.
Ethics training (including examples of ethical and unethical procedures and fraud detection techniques)	
Ethics program (e.g., reporting procedures if suspected unethical procedures)	
Laboratories " <u>must</u> " have an ethics policy and implement a fraud detection and deterrence policy/program, including use of data validation and verification techniques.	
If a laboratory employee suspects that fraudulent behavior is occurring, they "must" follow	

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reporting procedures established by their State; States should communicate these procedures	
to their laboratories. States "must" also advise laboratories as to the appropriate State point-of-	
contact should they have further questions related to suspected fraud. (Supplement #1, EPA	
815-F-08-006. June 2008)	•

#### Additional Suggestions:

- \* Page numbers of quality system documents should be of the type "page x of y".
- \* QA Plan/Manual (QAP/QAM) needs a reference section to list SOPs.
- \* The QA documents have revisions and typed names of those reviewing/approving. It is suggested that an "effective date" be added and that signatures be included to fully document approval/s.

#### **Additional Information:**

A. Please complete the following table indicating the approximate SDWA analytical workload by method:

		· /
Microbiology	Appx. # Samples/Yr.	Appx. % of Lab Workload/Yr.
Method		
SM9223B	(5,900 - )	57%
Ex. 5 - Delil	harativa	
Ex. o - Dem	oeralive	)
		i

B. Are drinking water samples, which are not SDWA compliance samples, analyzed with the same procedures and QC checks

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as samples required by SDWA? YES, with the exception of holding time. We will analyze samples from private household wells up to 48 hours from collection.

- C. Please provide the following documents (if possible in electronic format):
  - 1. Most current version of the laboratory quality manual and SOPs that apply to microbiological analyses. If these have previously been forwarded to EPA, indicate the revision # and effective date.
  - 2. The laboratory reports, PT provider reports and supporting analytical data for all WS PT studies over the last three years for each method that will be reviewed during the on-site (scope of SDWA certification).
- D. Please complete the attached checklist from the drinking water certification manual.

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Element	Number	Yes	No	NA	Comments
1. PERSONNEL	<del></del>	<del></del>			
Supervisor/Consultant	1.1				
Does the supervisor of the microbiology laboratory have a bachelor's degree in microbiology, biology, or equivalent?		X ~			,
Has a supervisor with a degree in a subject other than those listed above had at least one college-level microbiology laboratory course in which environmental microbiology was covered?				X	
In addition, has the supervisor had a minimum of two weeks training at a Federal or State agency or academic institution in microbiological analysis of drinking water or 80 hours of on-the-job training in water microbiology at a certified laboratory, or other training acceptable to the State or EPA?		X			
If a supervisor is not available, and a waiver has not been granted as per Section 1.3, is a consultant with the same qualifications substituted?				X	
Can the laboratory document that the consultant is acceptable to the State, and present on-site frequently enough to satisfactorily perform a supervisor's duties?				X	
Can the laboratory supervisor demonstrate that all laboratory personnel have the ability to satisfactorily perform the analyses to which they are assigned?		X	manage of the second		,
Can the laboratory supervisor demonstrate that all data reported by the laboratory meets the required quality assurance and regulatory criteria?	·	X/			
Analyst (or equivalent job title)	1.2				

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Yes No NA Element Number **Comments** Does the analyst have at least a high school education, X a minimum of three months bench experience in water, milk or food microbiology, training in microbiological analysis of drinking water acceptable to the State (or EPA), and a minimum of 30 days on-the-job training under an experienced analyst? Has the analyst demonstrated acceptable results on X unknown samples before analyzing compliance samples? 1.3 Waiver of Academic Training Has the certification authority waived the need for the X above specified academic training for highly experienced analysts in this laboratory? Has the certification authority waived the need for the X above specified training for supervisors of laboratories associated with drinking water systems that only analyze samples from that system? If yes to either of the above, does the laboratory have a X copy of that written and signed waiver available for inspection? 1.4 Personnel Records Does the laboratory maintain personnel records on X., laboratory analysts that include academic background, specialized training courses completed, and types of microbiological analyses conducted? 2. LABORATORY FACILITIES Does the laboratory have facilities that are clean and X temperature and humidity controlled, and with adequate lighting at the bench tops? Does the laboratory maintain effective separation of X incompatible testing areas?

Does the laboratory control access where appropriate,

and minimize traffic flow through the work areas?

X

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Element	Number	Yes	Ņo	NA	Comments
Does the laboratory ensure that contamination does not adversely affect data quality?		X		/	
Does the laboratory have bench tops and floors that are easily cleaned and disinfected?		X /			
Does the laboratory have sufficient space for processing samples; storage space for media, glassware, and portable equipment; floor space for stationary equipment; and areas for cleaning glassware and sterilizing materials?		XV			
Does the laboratory have provisions for disposal of microbiological wastes?		χÝ			
3. LABORATORY EQUIPMENT AND SUPPLIES					
Does the laboratory have the equipment and supplies needed to perform the approved methods for which certification has been requested?		X			
pH meter **	3.1				
Are accuracy and scale graduations within ±0.1 units?	3.1.1	X			
Are pH buffer aliquots used only once?	3.1.2	χŸ			
Are electrodes maintained according to the manufacturer's recommendations?	3.1.3	X.			
QC Are pH meters standardized before each use period with pH 7.0 and either 4.0 or 10.0 standard buffers, whichever covers the desired pH of the media or reagent?	3.1.4	X			3 point 4,7,10
QC Are both the date and buffers used recorded in a logbook along with the analyst's initials?		X			,
QC Is the pH slope recorded monthly, after calibration?	3.1.5	X			
QC If the pH meter does not have a feature to automatically calculate the slope, but can provide in the pH in millivolts, is the formula in Section 3.1.5.1 used to calculate the slope?	3.1.5.1	í		ΧĽ	

# Ex. 5 - Deliberative

# Date: February 27, 2012 **Ex. 5 - Deliberative**

Element	Number	Yes	No	NA	Comments		
QC If the slope is below 95% or above 105%, are the manufacturer's instructions followed for meter or electrode maintenance and general cleaning?	3.1.5.2	X					
QC Are commercial pH buffer solutions dated when received and when opened?	3.1.6	X,					
QC Are pH buffer solutions discarded by the expiration date?		X					
Balance (top loader or pan)	3.2		_	amman.			
Does the balance have a readability of 0.1 g?	3.2.1	X			Ex. 5 - Deliberative		
Does the balance have a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg for a load of 10 g or less?	3.2.2	X					
QC Are the balances calibrated monthly using ASTM Class 1, 2, or 3 weights (minimum 3 traceable weights which bracket laboratory weighing needs, with a readability of 0.1 g)?	3.2.3	X					
QC Are non-reference weights calibrated every six months with reference weights?				X			
QC Are calibrations recorded in a logbook with the initials of the individual performing the calibration?		X					
QC Are correction values on file and used?				χν			
QC Are reference weights re-certified every five years?		X				1	
QC Are damaged or corroded weights replaced?		X					
QC Are service contracts or internal maintenance protocols and maintenance records available?	3.2.4	X			Ex. 5 - D	eliberative	
QC Is maintenance, calibration, and cleaning conducted at least annually by a qualified independent technician, unless the need is modified or waived by the certification officer?		X V					
Temperature Monitoring Device	3.3						

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Element	Number	Yes	No	ŅA	Comments
Are glass, dial, or electronic thermometers graduated in 0.5°C increments (0.2°C increments for tests which are incubated at 44.5°C) or less, except as noted for hot air ovens (Section 3.6.1) and refrigerators (Section 3.9.1)?	3.3.1	X			
Does observation of glass thermometers indicate no separation in fluid columns?				X.	
Are only dial thermometers which can be adjusted used?				X	
QC Are glass and electronic thermometers calibrated annually and dial thermometers quarterly at the temperature used, against a NIST-traceable reference thermometer or one that meets the requirements of NBS Monograph SP 250-23?	3.3.2	X			
QC Are both the calibration factor and calibration date indicated on the thermometer?		X			

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Element	Number	Yes	No.	NA	Comments
QC Is the following calibration information recorded in a QC record book?  Serial number of the laboratory thermometer Serial number of the NIST-traceable thermometer (or other reference thermometer)  Temperature of the laboratory thermometer  Temperature of the NIST-traceable thermometer (or other reference thermometer)  Correction (or calibration) factor  Date of check  Analyst's initials		X			
QC Is the thermometer discarded if it differs by more than 1°C from the reference thermometer?	3.3.3	X			<u> </u>
<b>QC</b> Are reference thermometers recalibrated at least every five years?		X/	_		
QC Is reference thermometer calibration documentation maintained?		Χν			Ex. 5 - Delibera
QC Are continuous recording devices used to monitor incubator temperature recalibrated at least annually, using a reference thermometer that meets the specifications noted in Section 3.3.2?	3.3.4			X	
Incubator Unit	3.4				
Do incubator units have an internal temperature monitoring device and maintain a temperature specified by the method used, usually $35 \pm 0.5$ C and $44.5 \pm 0.2$ C?	3.4.1	X/			
For non-portable incubators, are thermometers placed on top and bottom shelves of the use area and immersed in liquid as directed by the manufacturer (except for electronic thermometers)?		X			

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Element	Number	Yes	No	NA	Comments	
When aluminum block incubators are used, do culture dishes and tubes fit snugly?				X		
QC Is the calibration-corrected temperature recorded for each thermometer being used at least twice per day during each day the incubator is in use?	3.4.2	X <sub>V</sub>				
QC Are these readings separated by at least four hours?		X				
QC Does the documentation include the date and time of reading, temperature, and technician's initials?		X				
If a circulating water bath is used, is it equipped with a gable cover to ensure an incubation temperature of $44.5 \pm 0.2$ C?		X		. •		
Autoclave	3.5				_	
Does the autoclave have an internal heat source, a temperature gauge with a sensor on the exhaust, a pressure gauge, and an operational safety valve?	3.5.1	X				
Can the autoclave maintain a sterilization temperature during the sterilizing cycle and complete an entire cycle within 45 minutes when a 12-15 minute sterilization period is used?		X			Ex. 5 - Del	lihorativo
Does the autoclave depressurize slowly enough to ensure that media will not boil over and bubbles will not form in inverted tubes?		X			LX. 3 - Dei	iberative

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	1		Γ		<u> </u>	n
Element	Number	Yes	No	NA	Comments	
QC Is the following information recorded each time the autoclave is used?  Date  Contents  Sterilization time and temperature  Total time in the autoclave  Analyst's initials  Ex. 5 - C	3.5.3 Delibei	x ⁄ati	ve			
QC Are copies of the service contracts or internal maintenance protocols and maintenance records kept?		Χν	/. /			
QC Is maintenance conducted at least annually?		x'			·	
QC Is a record of the most recent service performed on file and available for inspection?	TH!	x/				
QC Is a maximum-temperature-registering thermometer, electronic temperature readout device, or continuous recording device used each autoclave cycle to ensure that the proper temperature was reached?	3.5.4	X ~				
QC Is the temperature recorded?		X/				
QC Is overcrowding avoided?		X				W
QC Are spore strips or spore ampules used monthly as bioindicators to confirm sterilization?		*				
QC Are automatic timing mechanisms checked quarterly with a stopwatch or other accurate timepiece or time signal, and the results recorded and initialed?	3.5.5	Ϋ́			Ex. 5 - De	liberative
Are autoclave door seals clean and free of caramelized media?	3.5.6	X			,	I
Are autoclave drain screens cleaned frequently and debris removed?		X	/	·		
Hot Air Oven	3.6			$\mathbf{X}^{\mathbf{Z}}$		
Does the oven maintain a stable sterilization temperature of 170°-180°C for at least two hours?	3.6.1					
Is overcrowding avoided?	That I say					

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Element		Number	Yes	No	NA	Comments
Is the oven thermometer graduated in 10°C is ordess, with the bulb placed in sand during the						
QC Is the following information recorded to cycle?  Date Contents Sterilization time and temperature Analyst's initials	for each	3.6.2				
QC: Are spore strips used monthly to confine sterilization?	m .	3.6.3				
Colony Counter		3.7				. :
Is a dark field colony counter used to count Heterotrophic Plate Count colonies?			X			
Conductivity Meter		3.8				
Are meters suitable for checking laboratory is grade water and readable in units of either micromhos/cm or microsiemens/cm?	reagent-	3.8.1	X/			
QC Is the meter calibrated at least monthly following the manufacturer's recommendation using an appropriate certified and traceable 1 standard?	ons and	3.8.2	X			
QC If the meter cannot be calibrated as not is the cell constant determined at monthly in using a method in <i>Standard Methods</i> , Section	tervals				X	<i></i>
Is an in-line unit that cannot be calibrated use check reagent-grade water?	ed to	3.8.3		,	X	
Refrigerator		3.9				
Does the refrigerator maintain a temperature 5°C?	of 1°-	3.9.1	X			
Is the refrigerator thermometer graduated in 1°C increments and the thermometer bulb imliquid?	I	,	x/			

Element	Number	Yes	No	NA	Comments
QC On days the refrigerator is in use, and the laboratory is staffed, is the calibrated-corrected temperature recorded at least once per day?	3.9.2	X			•
Inoculating Equipment	3.10				
Are sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips used?		X/		·	
Are wood applicator sticks, if used, sterilized by dry heat?				X,/	
Are metal inoculating loops and/or needles made of nickel alloy or platinum?				x/	
Membrane Filtration (MF) Equipment	3:11			χÝ	
Are MF units made of stainless steel, glass, porcelain or autoclavable plastic?	3.11:1				
Are they scratched corroded, or leaking?					
QC-If graduations on clear or plastic funnels are used to measure sample volume, is their accuracy, checked with a Class B graduated cylinder or better (or other Class B glassware) and a record of this calibration check retained?	3.11.2				
Is a 10x to 15x stereo microscope with a fluorescent light source used to count sheen colonies?	3:11:3				
Are the membrane filters approved by the manufacturer for total coliform water analysis?	3.11.4				
Are membrane filters to be used cellulose ester, white; gridmarked; 47 mm diameter, and 0.45 µm pore size?					
If alternate pore sizes are used, does the manufacturer provide performance data equal to or better than the 0.45 µm pore size?					
Are membrane filters and pads purchased presterilized or autoclaved for 10 minutes at 121 °C before use?					

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Element	Number	Yes	No	NA	Comments
QC: Is the lot number for membrane filters and the date received recorded?	3.11.5				
QC - Are the membranes checked to see that they are not brittle or distorted?					
QC Are the manufacturer's specification/certification sheets available?	***				
Are the forceps blunt and smooth-tipped without corrugations on the inner sides of the tips?	3.11.6				
Culture Dishes (loose or tight lids)	3.12				
Are presterilized plastic or sterilizable glass culture dishes used?	3.12.1	X/			
Is the sterility of the glass culture dishes maintained by placement in stainless steel or aluminum canisters or a wrap of heavy aluminum foil or char-resistant paper?				X	
Are loose-lid petri dishes incubated in a tight-fitting container with a moistened paper towel?	3.12.2	X	<u> </u>		
Are opened packs of disposable culture dishes resealed between use periods?	3.12.3	X		-	
For membrane filter methods, are culture dishes of an appropriate size to allow the transfer of a single membrane per plate?	3.12.4	. !		X	,
Pipets	3.13				
Are glass pipets sterilized and maintained in stainless steel or aluminum canisters or wrapped individually in char-resistant paper or aluminum foil?	3.13.1		·	X	/
Do pipets have legible markings and are they not chipped or etched?	3.13.2	X	<i>,</i>		
Are opened packs of disposable sterile pipets resealed between use periods?	3.13.3	X,			
Are pipets delivering volumes of 10 mL or less accurate to within a 2.5% tolerance?	3.13.4	X			

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Element	Number	Yes	No	NA	Comments
Are calibrated micropipetters used with sterile tips?	3.13.5	χi⁄			
Are micropipetters calibrated annually and adjusted or replaced if the precision or accuracy is greater than 2.5%?		X/			
Glassware and Plasticware	3.14				
Is the glassware made of borosilicate glass, or other corrosion-resistant glass, and free of chips and cracks?	3.14.1	X V			]
Are markings on graduated cylinders and pipets legible?		X		e *	
Are plastic items clear and nontoxic to microorganisms?		X			
QC Are the graduated cylinders used for measurement of sample volumes, or other precalibrated containers that have clearly marked volumes used in lieu of graduated cylinders, accurate to within a 2.5% tolerance?	3.14.2	X		· ·	
Are culture tubes and containers containing fermentation medium of sufficient size to contain medium plus sample without being more than three quarters full?	3.14.3	X			
Are tube closures made of stainless steel, plastic, aluminum, or screw caps with nontoxic liners?	3.14.4	X			
Are cotton or foam plugs used?				XΥ	
Sample Containers	3.15				
Are sample containers wide-mouth plastic or non-corrosive glass bottles with non-leaking ground glass stoppers or caps with nontoxic liners, sterile plastic bags containing sodium thiosulfate, or other appropriate sample containers?	3.15.1	X		Ex.	5 - Deliberativ
Is sample container capacity at least 120 mL (4 oz) to allow at least a 1-inch head space?		$\begin{pmatrix} x \end{pmatrix}$			150 mh
Are glass stoppers covered with aluminum foil or char- resistant paper for sterilization?	3.15.2			X	

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Element	Number	Yes	No	NA	Comments
Are unsterilized glass and plastic bottles sterilized by autoclaving or, alternatively, by dry oven for glass bottles?	3.15.3	,		X	
Are empty containers moistened with several drops of water before autoclaving to prevent an "air lock" sterilization failure?				x /	
If chlorinated water is to be analyzed, is sufficient sodium thiosulfate added to the sample bottles before sterilization to neutralize any residual chlorine in the water sample?	3.15.4	X		7	
Ultraviolet Lamp (if used)	3.16	,			
Is the germicidal unit disconnected monthly and the	3.16.1	<u> </u>	Ι.Λ	*	Ex. 5 - Deliberative
lamp cleaned by wiping with soft cloth moistened with ethanol?	Ex. 5 -	Delib	erati	ve	
Is the longwave unit used for fluorometric tests kept clean?		X			
QC Is the germicidal unit tested quarterly with a UV light meter or agar spread plate?	3.16.2			X	
QC Is the lamp replaced if it emits less than 70% of its initial output or if an agar spread plate containing 200 to 250 microorganisms, exposed to the UV light for two minutes, does not show a count reduction of 99%?				X	
Spectrophotometer or colorimeter (if used)	3.17			χ̈́	
Are wavelengths in the visible range?	3.17.1				
QC Is a calibration standard and a method-specific blank analyzed every day the instrument is used, prior to sample analysis?	3.17.2				
QC : Is this calibration standard obtained from an outside source?					
QC : Does the calibration standard give a reading in the desired absorbance range?					

Sample Containers

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Element	Number	Yes	No	NA	Comments
4. GENERAL LABORATORY PRACTICES					
Are laboratory personnel aware of general and customary safety practices for laboratories?		X			
Does the laboratory have a safety plan available?		X,			
Does the laboratory keep a copy, and follow the personal protection guidelines, of any material safety data sheet accompanying the receipt of a toxic material?		X			
Sterilization Procedures	4.1				
Does the laboratory follow the minimum times for autoclaving the materials listed below at 121°C?  Membrane filters and pads 10 min Carbohydrate containing media 12-15 min Contaminated test materials 30 min² Membrane filter assemblies 15 min Sample collection containers 15 min Individual glassware 15 min Dilution water blank 15 min Rinse water (0.5 - 1 L) 15-30 min²  **except where otherwise specified by the manufacturer**  **ine depends upon water volume per container and autoclave load**	4.1.1	X			
Are autoclaved membrane filters and pads and all media removed immediately after completion of the sterilization cycle?	4.1.2			X	
Is membrane filter equipment autoclaved before the beginning of a filtration series?	4.1.3			X/	
If a UV light (254 nm) is used to sanitize equipment after initial autoclaving for sterilization, are all supplies presterilized?	4.1.4			X	

4.2

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QC Is at least one sample container selected at random from each batch of sterile sample bottles, or other containers (or lot of commercially available sample containers), and the sterility confirmed by adding 25 mL of a sterile non-selective broth, incubating at 35±0.5°C, and checking for growth after 24 and 48 hours?		X ~		
QC Are these results recorded?		x		
QC If growth is detected, is the entire batch resterilized?		X		
Reagent-Grade Water	4.3	•		
Does the laboratory only use satisfactorily tested reagent water from stills or deionization units to prepare media, reagents, and dilution/rinse water for performing microbial bacteriological analyses?	4.3.1	X		

Element	Number	Yes	No	NA	Comments
C Is the quality of reagent water tested and does it meet the following criteria?	4.3.2	X			
Conductivity >0.5 megohms resistance Monthly* or <2 micromhos/cm (microsiemens/cm) at 25°C					
Pb, Cd, Cr, Not greater than 0.05 mg/L Annually Cu, Ni, Zn per contaminant. Collectively no greater than 0.1 mg/L		·			
Total chlorine < 0.1 mg/L Monthly residual*					
Heterotrophic <500/mL CFU/mL* Monthly plate count*					
Bacteriological Ratio of growth rate Annually quality of 0.8 to 3.0 reagent water*	( h)	A			
*See Section 4.3.2 for footnotes					

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Element	Number	Yes	No	NA	Comments
Dilution/Rinse Water	4.4				
Is stock buffer solution or peptone water prepared as specified in <i>Standard Methods</i> , Section 9050C?	4.4.1	X	·		
Are stock buffers autoclaved or filter-sterilized?	4.4.2	X		/	
Are these containers labeled, dated, and refrigerated?		X			
Are stored stock buffers free from turbidity?		X		1/	
QC Is each batch (or lot, if commercially prepared) of dilution/rinse water checked for sterility by adding 50 mL of water to 50 mL double strength non-selective broth, incubating at 35± 0.5°C, and checking for growth after 24 hours and 48 hours?		X	·		
QC Are these results recorded?		X		;	
QC Is the batch/lot discarded if growth is detected?		X			
Glassware Washing	4.5		<i>)</i>		
Is distilled or deionized water used for the final rinse?	4.5.1	X.			·
Is laboratory glassware washed with a detergent designed for laboratory use?	4.5.2	X_/			
QC Is the glassware inhibitory residue test performed before the initial use of a washing compound and whenever a different formulation, or washing procedure is used?	4.5.3	X			
QC Are these results recorded?		X			
QC Is each batch of dry glassware used for microbial analysis spot-checked for pH reaction using 0.04% bromthymol blue (or equivalent pH indicator) and the color reaction recorded?	4.5.4	X			Ex. 5 - Deliberativ
5. ANALYTICAL METHODOLOGY				•	
General	5.1				

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Element	Number	Yes	No	NA	Comments
For compliance samples, does the laboratory use only the analytical methodologies specified in the Total Coliform Rule (TCR), the Surface Water Treatment Rule (SWTR), and the Groundwater Rule (GWR)?	5.1.1	X V			
Is the laboratory certified for all analytical methods it uses for compliance purposes?	5.1.2	X			
At a minimum, is the laboratory certified for one total coliform method and one fecal coliform or <i>E. coli</i> method?		X ✓			
Is the laboratory certified for a second total coliform method if one method cannot be used for some drinking waters?		x V			
For a laboratory that enumerates heterotrophic bacteria for compliance with the SWTR, is the laboratory certified for either the Pour Plate Method or the SimPlate method for heterotrophic bacteria?				X V	
Are water samples shaken vigorously at least 25 times before analyzing?	5.1.3	X		·	
QC If dilution buffer is used, does the laboratory check the buffer volume in one dilution bottle of each batch or lot?	5.1.4	X		/	
QC For a 90-mL or 99-mL volume, is the tolerance ±2 mL?		$\mathbf{x}_{\mathbf{v}}$			
Does the laboratory analyze a 100-mL sample volume for total coliforms in drinking water?	5.1.5	X			
Media (or defined substrate)	5.1.6			/	
Are dehydrated media stored in a cool dry location and discarded by the manufacturer's expiration date?	5.1.6.1	X			
Is caked or discolored dehydrated media discarded?		X			

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Element	Number	Yes	No	NA	Comments
QC For media prepared in the laboratory is the following information recorded?  Date of preparation  Type of medium  Lot number  Sterilization time and temperature  Final pH (after sterilization)  Technician's initials	5.1.6.2	X			:

QC For media prepared commercially is the following recorded for each lot?  Date received  Type of medium  Lot number  pH verification	5.1.6.3	X			
<b>QC</b> Are media prepared commercially discarded by manufacturer's expiration date?		X			
QC Is each new lot of dehydrated or prepared commercial medium and each batch of laboratory-prepared medium checked before use for sterility and with positive and negative culture controls?	5,1.6.4	X			
QC Are these results recorded?		X			
QC For laboratories using commercially prepared media with manufacturer shelf-lives of greater than 90 days, are positive and negative controls run each quarter, in addition to that noted above?		X			
QC Are these results recorded?		X/	/		
QC For control organisms, are stock cultures periodically checked for purity and the results recorded, or are commercially available disks impregnated with the organism used?		X		•	·

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Element	Number	Yes	No	NA	Comments
If prepared medium is stored after sterilization, is it maintained in the dark as follows?  poured plates 1°-5°C 2 weeks broth in containers with 1°-30°C2 weeks loose-fitting closures broth in tightly closed 1°-30°C 3 months containers	5.1.6.5	X			
QC Does the laboratory perform parallel testing between a newly approved test and another EPA-approved procedure for enumerating total coliforms for at least several months and/or several seasons to assess the effectiveness of the new test for the wide variety of water types submitted for analysis? Recommended.	5.1.7	X			
Does the laboratory perform the approved methods listed in this section for the TCR, SWTR, and/or GWR?	5.1.8	X_		÷	
Fermentation broth methods	5.2				
General	5.2.1				
Is the water level of the water bath above the upper level of the medium in the culture tubes?	5.2.1.1	·X~			/
If a Dri-bath incubator is used, is the specified temperature requirement maintained in all tube locations used?	5.2.1.2			X	
Multiple Tube Fermentation Technique (for detecting total coliforms in drinking water and enumerating total coliforms in source water)	5.2.2	X		•	
For drinking water samples, is the total sample volume of 100 mL used for each test?'	5.2.2.1	X	/		
For source water samples, are at least 3 series of five tubes each with appropriate sample dilutions used?	5.2.2.2			X	
Media	5.2.2.3				

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Element	Number	Yes	No	NA	Comments
Is lauryl tryptose broth (LTB) used in the presumptive test and 2% brilliant green lactose bile broth (BGLBB) in the confirmed test?	5.2.2.3.1	X V			
If lactose broth (LB) is used in lieu of LTB, has the laboratory conducted at least 25 parallel tests between this medium and LTB using the waters normally tested?	,			X	
Has this comparison demonstrated that the false-positive rate and false-negative rate for total coliforms, using LB, is less than 10%?				X	
Is this comparison information documented and the records retained?				X/	/
Is the final pH of LTB medium $6.8 \pm 0.2$ ?		X/			
Is the final pH of 2% BGLBB $7.2 \pm 0.2$ ?		X.⁄			
Is the test medium concentration adjusted to compensate for the sample volume so that the resulting medium after sample addition is single strength?	5.2.2.3.2	X V			
If a single 100-mL sample volume is used, is the inverted vial replaced with an acid indicator (bromcresol purple)?		X			
Is the medium autoclaved at 121°C for 12-15 minutes?		Χ -			
Is the sterile medium in tubes examined to ensure that the inverted vials, if used, are free of air bubbles and are at least one-half to two-thirds covered after the water sample is added?	5.2.2.3.3	X			
Is the inoculated medium incubated at 35±0.5°C for 24±2 hours?	5.2.2.4	X			
If no gas or acid detected, is the inoculated medium incubated for another 24 hours for a total incubation time of 48±3 hours?		X			
Is each 24- and 48-hour tube that has growth or is gaspositive or acid-positive confirmed using 2% BGLBB?	5.2.2.5	X	r'		

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Element	Number	Yes	No	NA	Comments
For drinking water samples, is each total coliform-positive sample tested for the presence of either fecal coliforms or <i>E. coli</i> ?	5.2.2.6	X	<i>j</i>	,	·
Invalidation of total coliform-negative samples	5.2.2.7	χ./			
For drinking water samples, are all samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, invalidated?	5.2.2.7.1	X	,		
Does the laboratory then collect, or request that the system collect, another sample within 24 hours from the same location as the original invalidated sample?		X/			
Although not required before invalidation, does the laboratory perform a confirmed test and/or a fecal coliform/ <i>E. coli</i> test on the total coliform-negative culture to check for coliform suppression?		X/			
And if the confirmed test is total coliform-positive or fecal coliform/ <i>E. coli</i> -positive, does the laboratory report the sample as such?		X			
If the follow-up test is total coliform-negative, does the laboratory invalidate the sample?		x'			
For source water samples, are all samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production in LTB or LB invalidated?	5.2.2.7.2		-	X V	
Does the laboratory collect, or request that the system collect, another sample from the same location as the original invalidated sample?	,			X	
Although not required before invalidation, does the laboratory perform a confirmed test on the total coliform-negative culture and, if the confirmed test is total coliform-positive, is the MPN reported?	·			X	
If the confirmed test is total coliform-negative, is the sample invalidated?				X <sub>/</sub>	
Presence-Absence (P-A) Coliform Test (for detecting total coliforms in drinking water)	5.2.3			X	

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Element	Number	Yes	No	NA	Comments
Medium	5.2.3.1			Χv	
When six-times formulation strength medium is used, is it filter-sterilized rather than autoclaved?	5.2.3.1.1			X	
Is the medium autoclaved for 12 minutes at 121°C?	5.2.3.1.2			. X ,	
Is the total time in the autoclave less than 30 minutes?				Χ :	
Are the bottles placed in the autoclave with space between them?				$X_{\nu}$	
Is the final pH of the medium 6.8±0.2?				Χ -	
If the prepared medium is stored, is it maintained in a culture bottle at 1°-30°C in the dark for no more than 3 months?	5.2.3.1.3			X	/-
Is the stored medium discarded if evaporation exceeds 10% of original volume?				X	
Is a 100-mL sample inoculated into a P-A culture bottle?	5.2.3.2			X	
Is the sample/medium incubated at 35±0.5°C and observed for yellow color (acid) after 24 and 48 hours?	5.2.3.3			X	
Are yellow cultures confirmed in BGLBB and a fecal coliform/ <i>E. coli</i> test conducted?	5.2.3.4			Χν	
Are all samples which produce a non-yellow turbid culture in P-A medium invalidated?	5.2.3.5			X	
Does the laboratory collect, or request that the system collect, another sample from the same location as the original invalidated sample?				X Z	
Although not required before invalidation, does the laboratory perform a confirmed test on the total coliform-negative culture and/or a fecal coliform/ <i>E. coli</i> test and, if the confirmed test is total coliform-positive, is the sample reported as such?				Χν	
If the confirmed test is total coliform-negative, is the sample invalidated?				X	

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Element	Number	Yes	No	NA	Comments
Fecal Coliform Test (using EC Medium for fecal coliforms in drinking or source water, or A-1 Medium for fecal coliforms in source water only)	5.2.4	X			
EC Medium	5.2.4.1	Χν			
Is EC medium used to test a total coliform-positive culture for fecal coliforms under the Total Coliform Rule?	5.2.4.1.1	X			
Is each total coliform-positive culture transferred from a presumptive tube/bottle, or each presumptive total coliform-positive colony (unless a cotton swab is used), to at least one tube containing EC Medium with an inverted vial?		X			.:
Is EC medium used to enumerate fecal coliforms in source water, in accordance with the SWTR?	5.2.4.1.2			X	
When conducting a MTF test, are three sample volumes of source water with five or ten tubes/sample volume used?				X	
Is a culture from each total coliform-positive tube transferred to a tube containing EC Medium with an inverted vial?				X	
Is EC Medium autoclaved at 121°C for 12-15 minutes?	5.2.4.1.3	X			
Is the final pH of EC medium 6.9±0.2?		Χν			
Are the inverted vials examined to ensure that they are free of air bubbles and at least one-half to two-thirds covered after the sample is added?	5.2.4.1.4	X			
Is EC Medium incubated at 44.5±0.2°C for 24±2 hours?	5.2.4.1.5	Χ×	/ · ]		
Is any amount of gas detected in the inverted vial of a tube that has turbid growth considered a fecal coliform-positive test, regardless of the result of any subsequent test on that culture?	5.2.4.1.6	X			
A-l-Medium	5.2.4.2			X.V	

Element	Number	Yes	No	NA	Comments
If A=1 Medium is used is it used to enumerate only fecal coliforms in source water in accordance with SWTR, and not for drinking water samples?	5:2.4:2:1			x /	
Are three sample volumes of source water used in a five-or ten-tube/sample volume format?				Χ×	
Is A-1 Medium autoclaved at 121°C for 10 minutes?	5.2.4.2.2			ر X	
For A-1 Medium, is the final pH 6.9±041?				X-/	
Are inverted tubes examined to ensure that they are free of air-bubbles?	5.2:4.2.3 ***********************************			X,	
Is A-1. Medium incubated at 35±0.5°C for three hours, and then incubated at 44.5±0.2°C for 21±2 hours?	5:2:4:2.4			Χ	
Are loose-cap tubes stored in the dark at room temperature for no longer than two weeks; or in tightly closed screw-cap tubes in the dark at <30°C for no longer than three months?	5.2.4.2.5			X=; //	
is any amount of gas detected in the inverted vial of a a tube with turbid growth considered a fecal coliform-positive test?	5.2.4.3			X /	
Azide dextrose medium (for detecting fecal streptococci in ground water)	5.2.5			ΧŻ	
For testing 100-mL samples, is triple strength (3X) formulation in a culture bottle prepared and then autoclaved at 121°C for 15 minutes?	5.2.5.1			X	
Is medium final pH 7.2±0.2?				X:/	
Is a 100-mE water sample added to the sterilized medium and incubated at 35±0.5°C?	5.2.5:2			X	
Is the culture checked for turbidity after 24±2 hours?	5.2.5.3			X' /	
If turbidity is not observed, is the culture reincubated and checked again after a total incubation period of: 48±3 hours?				X: /	

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Element	Number	Yes	No	NA	Comments
Are turbid cultures confirmed as fecal streptococci by streaking a portion of the broth onto bile esculin agar (BEA) or bile esculin azide agar (BEAA)?	5.2.5.4			X	
Are BEA and BEAA autoclaved at 121°C for 157 minutes?	5:2:5.5			x /	
Is the final pH 6:6±0.2 for BEA and 7:1±0.2 for BEAA?				X /	
After streaking, are plates incubated at 35±0.5€ for 48 hours?	5.2.5.61			x ½	
Are the brownish-black colonies with brown halos on BEA or BEAA used as confirming the presence of fecal streptococci?	5.2.5.7°			X	
If required, does the laboratory perform an enterococci test by transferring one or more fecal streptococci colonies to brain heart infusion broth supplemented a with 6.5% NaCl and incubating the culture at 35±0:5C for 48 hrs?				X	
Enzyme (chromogenic/fluorogenic) substrate tests	5.3				
General	5.3.1				
For detecting total coliforms and <i>E. coli</i> in drinking water by an enzyme substrate test, does the laboratory use one of the following: MMO-MUG test (Colilert), Colisure test, E*Colite test, Readycult Coliforms 100 Presence/Absence Test, Fluorocult LMX test, or Colitag test?	5.3.1.1	X			,
For enumerating total coliforms in source waters by an enzyme substrate test, does the laboratory use the Colilert test?	·	X ✓	l		
If a laboratory uses a fermentation method to detect total coliforms in drinking water, and the sample is total coliform-positive, does the laboratory transfer the positive culture to the EC+MUG test to detect <i>E. coli</i> , but not to any other enzyme substrate test medium in Section 5.3?				X	

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Element	Number	Yes	No	NA	Comments
Media	5.3.1.2				
Does the laboratory purchase media from a commercially available source only, and not prepare media from basic ingredients?	5.3.1.2.1	X			
Are media kept protected from light?	5.3.1.2.2	ΧV		_	
Is each lot of medium checked for fluorescence before use with a 365-366-nm ultraviolet light with a six watt bulb?	5.3.1.2.3	X			
If medium exhibits faint fluorescence, is another lot used that does not fluoresce?		X			
If samples plus medium exhibit color changes before incubation, is the medium discarded and another lot of medium used?	5.3.1.2.4	X			
Are glass and plastic bottles and test tubes checked before use with a 365-366-nm ultraviolet light source with a 6-watt bulb to ensure that they do not fluoresce?	5.3.1.3	X			
If they fluoresce, does the laboratory use another lot of containers that does not fluoresce?		X		-	
If a Whirl-Pak® bag is used to incubate the Colilert or Colitag medium or any other medium which changes to a yellow color to indicate a positive result, is a type used that has a barrier (e.g., B01417) to prevent gaseous emissions to other Whirl-Pak® bags during incubation?	5.3.1.4			X	
<b>QC</b> If a small air-type incubator is used, are samples brought to room temperature before incubation?	5.3.1.5	-		X	
If a water bath is used, is the water level above the upper level of the medium?	5.3.1.6	X		1	
For E. coli testing, are all total coliform-positive samples placed under a UV lamp (365-366 nm) in a darkened area?	5.3.1.7	X			

Element	Number	Yes	No	NA	Comments
Does the laboratory refrain from using the enzyme substrate test to confirm a presumptive total coliform-positive culture in a fermentation broth or on a membrane filter?	5.3.1.8	X			
Does the laboratory invalidate any sample that produces an atypical color change (in the absence of a yellow color) and then collect, or request that the system collect, another sample from the same location as the original invalidated sample?	5.3.1.9	X		•	
Does the laboratory use another method to test the second sample?		X			
Is the reference comparator provided by the manufacturer discarded by the manufacturer's expiration date?	5.3.1.10	x/			,
Criteria for specific media	5.3.2				_
For the Colilert test, are samples incubated at 35±0.5°C for 24-hours?	5.3.2.1	X			
Is a sample with a yellow color in the medium equal to or greater than reference comparator recorded as total coliform-positive?		X			
Is a sample with a yellow color lighter than comparator incubated for another four hours but no longer than 28 hours total?		X V			
Is a sample with a yellow color lighter than the comparator after 28 hours of incubation recorded as total coliform-negative?		X	/		
Are coliform-positive samples that fluoresce under a UV light marked as <i>E. coli</i> -positive?		X_	/		
For the Colilert-18 test, are samples incubated for 18 hours (up to 22 hours if the sample after 18 hours is yellow, but lighter than the comparator)?		x V			

Element	Number	Yes	No	NA	Comments
For enumerating total coliforms in source waters, does the laboratory use the Colilert test, a 5- or 10-tube configuration, Quanti-Tray, or Quanti-Tray 2000 for each sample dilution tested?	5.3.2.1.1	x/			·
When dilution water is used, is it either sterile deionized or sterile distilled water, not buffered water?		X/			
<b>QC</b> If the Quanti-Tray or Quanti-Tray 2000 test is used, is the sealer checked monthly by adding a dye to the water?	5.3.2.1.2	x V			
For the Colisure test, are samples incubated at 35±0.5°C for 24-48 hours?	5.3.2.2			х ; - V	
If the medium changes from a yellow color to a red/magenta color is the sample noted as total coliform-positive?				× ν	
Is a coliform-positive sample that fluoresces under a UV light marked as <i>E-coli</i> -positive?				x · Y	
For the E*Colite test, is the sample incubated at > ====35±0.5€ for 28 hours?	53.2.3			×	
If the medium changes from a yellow color to a blue or blue-green color, or a blue color in the corners of the bags is the sample marked as total coliform-positive?				×	
If the medium fluoresces under a UV light, is the sample considered as $\hat{E}$ coli-positive?					
If fluorescence is not observed; is the sample reincubated for an additional 20 hours (for a total incubation time of 48 hours) and checked again for fluorescence?				ΧV	
If the medium becomes red in color, is the sample discarded and another sample requested?				·X· ·V	
For the Readycult Coliforms 100 Presence-Absence test; are the contents of a snap pack added to a 100-mL sample and then incubated at 35±0.5°C for 24±1 hours?	5.3.2.4			×.	/

Element	Number	Yes	No	NA	Comments
If the medium changes color from a slightly yellow color to blue-green; is the sample marked as coliform-positive?				X * <b>/</b>	
If the medium fluoresces a bright light-blue color when subjected to long wave UV (365-366 nm) light; is the sample marked as $E*coli$ -positive?				x Z	
For the Fluorocult LMX test; is the medium added to purified water, mixed; and the mixture then boiled to dissolve the medium completely in the water?	53.2.5			X:	
Are 100-mL aliquots transferred to 250-mL bottles and then autoclaved for 15 minutes?				Χ	
Are the autoclaved bottles cooled before adding the 100-mL water sample?				X: V	
Is the $E$ coli/Coliform Supplement not added to the medium?				x.	
Is the sample then incubated at 35±0.5°C for 24±1 hours?				X	
If the medium changes color from a slightly yellow color to blue-green; is the sample marked as coliform-positive?				X. نز	
If the medium fluoresces a bright light-blue color, when subjected to long wave UV (365-366 nm) light; is the sample marked as E. coli-positive?				X,	at unit of the
For the Colitag test, are samples incubated at 35°±0.5°C for 24±2 hours?	5.3.2.6				NEW METHOD
If the medium changes to a yellow color, is the sample marked as coliform-positive?		<b>V</b>			
If the medium fluoresces under a UV light, is the sample marked as <i>E. coli</i> -positive?		<b>/</b>			/
EC Medium +: MUG (for detection of E. coli)	5.3.3			χ÷Υ	Transfer was a

Element	Number	Yes	No	NA	Comments
If EC medium+:MUG is used, is a total coliform- positive culture transferred from a presumptive tube/bottle or colony to this medium?	5.3.3:1				
Is the final pH of EC medium + MUG 6.9±0.2?	53.3.2				
Is the medium plus sample incubated at 44.5±0.20 for 24±2 hours and then tested for fluorescence?	5.3.3.4				
Enterolert test (for detection of enterococci in ground water)	5.3.4	X v	1		
Is the medium stored in the dark at 4-30°C until used?	5.3.4.1	Χį	/		
Is Enterolert reagent added to a 100-mL sample and the sample/medium incubated at 41°±0.5°C for 24-28 hours?	5.3.4.2	X ~			
Is fluorescence under a UV lamp used to indicate the presence of enterococci?		×			
Membrane Filter (MF) methods	5.4			χν	DROPPING?
General	5:4.1				
For source water samples (SWTR), do dilutions yield 20 to 80 total coliform colonies or 20 to 60 fecal coliforms for at least one dilution or volume?	5.4.1.1				
QC Is at least one membrane filter and filtration unit sterility check conducted at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter and testing for growth?	5.4:1.2				
QC If the control indicates contamination, does the laboratory reject all data from affected samples and request an immediate resampling?			A MASSAGE		
QC Does the laboratory consider a filtration series as ended when 30 minutes or more has elapsed between sample filtrations?					

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Element	Number	Yes	No	NA	Comments
Are filtration funnels rinsed after each sample filtration with two or three 20-30 mL portions of sterile rinse water to ensure that the entire sample is rinsed off the funnel onto the filter?	5413				
Are absorbent pads saturated with at least 2 mL of broth and the excess medium removed by "decanting" the plate?	5.4.1.4				
MF method for detecting total coliforms and E coli in drinking water, enumerating total coliforms or fecal coliforms in source water, and detecting E coli in ground water	5.4:2:32 2:32 2:32 3:32 3:32 3:32 3:32 3:3				
Media for total coliforms, fecal coliforms, and $E \sim coli$	5.4.2.1				
If either M-Endo agar or broth or M-Endo agar LES is used to detect total coliforms in drinking water or enumerating total coliforms in source water, is either the single step or the enrichment technique used?	5.42.1.1				
Is denatured ethanol used in the rehydration procedure?					
Iş the medium prepared in a sterile flask?					1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1
Is a boiling water bath or a constantly attended hot black with a stir bar used to bring the medium just to the boiling point but not boiled?					
Is the final for M-Endo medium pH 7.2±0:1 and the final pH for M-Endo Agar LES 7.2±0:2?					
Is M-Endo medium or M-Endo agar LES, incubated at 35°±0.5°C for 22-24 hrs?	(5.4.2.2)				
Are colonies with a metallic (golden) sheen recorded as presumptive total coliforms?	(5:4-2-2)				

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Element	Number	Yes	No	NA	Comments
If m=ColiBlue24 medium; is used to detect total coliforms and Excoli in drinking water, are the ampules of broth inverted 2-3 times to mix contents.	5.4.2.1.2				
before breaking and the contents then poured evenly over an absorbent pad?					
Are unopened refrigerated ampules stored in the dark?				7. A.	
Are unopened ampules discarded before the expiration date, or earlier if contamination is observed?					
Is the medium final pH 7.0±0.2?					
Is m=ColiBlue24 incubated at 35%±0/5%C for 24 hrs?	(5.4.2.2)				
Are red colonies recorded as total coliforms, and blue to purple colonies recorded as E. coli?	(5:4.2.2)				
If MI medium (with or without agar) is used to detect total coliforms and $E$ , $coli$ in drinking water or enumerate total coliforms in source water, is commercially prepared presterilized bottled MI agar or broth not autoclaved?	5.42.113				
Is this presterilaized bottled agar medium melted in a boiling water bath (or by other processes), recommended by the manufacturer), and care taken not to overheat the agar?					
Is the medium then cooled slightly and poured			F. 1.		
If dehydrated culture medium is used, is it prepared and autoclaved according to manufacturer's instructions?					T.
Is this agar medium cooled before adding freshly prepared, filter- sterilized cetsulodin, and then poured immediately into sterile plates?					
Is the final pH of MI agar 6.95±0.20 and the final pH of MI broth 7.05±0.20?					
Is MI medium incubated at 35°±0.5°C; for 24±2 hrs?	(5.4.2.2)				

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Element	Number	Yes	No	NA	Comments
Are fluorescent colonies under UV light recorded as total coliforms, and blue colonies under normal light recorded as E. coli?	(5.4.2.2)				
If Chromocult® Coliform Agar is used to detect total coliforms and E. coli in drinking water, is the agar medium autoclaved or overheated?	5.4.2.1.4				
Is the final pH of this medium 6.8±0.2?					
If a heavy background of heterotrophic bacteria is expected; is cefsulodin solution added to 1L of cooled-(43-50°C) medium as a solution of 10 mg cefsulodin dissolved in 2-mL deionized or distilled water?					
Is Chromocult incubated at 36°±1°C for 24±1 hrs?	(5.4.2.2)			ren e	
Are salmon to red colonies recorded as total coliforms, and dark-blue to violet colonies recorded as E. coli?	(5.4.2.2)				
If Coliscan® is used to detect total coliforms and E. coli in drinking water or enumerate total coliforms in source water is the manufacturer's protocol for reconstitution and antibiotic addition followed?	5.42.1.5				
Is the antibiotic, cefsulodin, overheated?					
Is the final pH of Coliscan agar 7.00±0.20?					
Is Coliscan incubated at 32°-37°C for 24-28 hrs?	(5.4.2.2)				a de la companya de l
Are pink-magenta colonies recorded as total coliforms, and purple-blue colonies recorded as E coli?	(5.4.2.2)				
If m-FC broth, with or without agar, is used to enumerate fecal coliforms in source water, is the medium autoclaved?	5.4.2.1.6 2				
Is m-FC broth just brought to the boiling point?					
Is the final pH of m-FC medium 7.4±0.2?					
Is m-FC broth incubated at 44.5°±0.2°C for 24±2 hrs?	(5.4.2.2)		44 7 1		
Are blue colonies recorded as fecal coliforms?	(5.4.2.2)				

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Element	Number	Yes	No	NA	Comments
Is the prepared medium refrigerated when stored and brought to room temperature before use?	5.4.2.1.7				
Are petri dishes containing medium stored in a plastic- bag or tightly closed container, and used within 2 weeks?					
Are plates with laboratory-prepared broth medium discarded after 96 hours; poured agar plates after 2 weeks; and ampuled broth discarded before the manufacturer is expiration date?					
Are the date and time of medium preparation : recorded?					
For invalidation of a total coliform-negative drinking water sample, are all samples resulting in confluent a growth or TNTC growth invalidated unless at least one total coliform colony is detected?	5.4.23				
If no coliforms are detected; is the sample recorded as sconfluent growth; or "TNTC" and an additional sample requested from the same sampling site?					
Does the laboratory perform a ventication test on the total coliform-negative culture before invalidation?					
If the verification test is total coliform-positive, does the laboratory report the sample as total coliform-positive?					
If the verification test is total coliform-negative, is the sample invalidated?					
For invalidation of source water samples (SWTR), where coliform density must be determined does the laboratory invalidate any sample that results in confluent growth or TNTC; even when total coliform or fecal coliform colonies are present?	5.4.2.4				

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Element	Number	Yes	No	NA	Comments
For drinking water samples on M-Endo type media, are all sheen colonies sup to a maximum of five, verified by using either LB or LTB and then 2% BGLBB or alternatively, by using a cytochrome	5.4.2.5				
oxidase and β-galactosidase procedure?  If no sheen colonies are observed; are up to five red questionable sheen colonies and/or red non-sheen colonies representing different morphological types venified?					
For drinking water samples, are total coliform-positive colonies tested for E. coli or fecal coliforms?	5.4.2.6				
When EC Medium or EC + MUG is used, are colonies transferred by employing one of the options specified by the Total Coliform Rule?					
When the swab technique is used, is a single swab used to inoculate a presumptive total coliform-positive sample into EC or EC+MUG first, LTB second, and BGLBB third?					
For source water samples, are the initial total coliform counts adjusted based upon verified data?	5.4.2.7				
QC For source water samples when two or more analysts are available; does each analyst count the total coliform or fecal coliform colonies on the same membrane monthly and do the counts agree within 10%?	5.4.2.8				
Nutrient Agar + MUG Test (for detection of E. coli in drinking water or ground water)	5.4.3				
Is the medium autoclaved at 121°C for 15 minutes?	5.4.3.1				
Is the final MUG concentration 100 μg/L?					
Is the final pH-of NA+, MUG 6.8±0.2?					
QC: Are positive and negative culture controls tested as stated in 5:1.6:4?	5.4.3.2				

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Element	Number	Yes	No	NA	Comments
QC: Are culture controls filtered or spot-inoculated onto a membrane filter on M-Endo broth or agar, or M-Endo agar LES; and incubated at 35±0.5°C for 24 hours?				A STATE OF THE STA	
QC Is the filter then transferred to NA + MUG and incubated at 35±0.5°C for another four hours?					
QC Are these results read and recorded?					
Is the membrane filter containing total coliform colonies transferred to the surface of the Nutrient Agar # MUG medium?	5.4.3.3				
Is the presence of each sheen colony marked on the petrodish lid with permanent marker, and the lid and base marked to realign the lid when removed?			<b>医一种一种</b>		
For the total coliform verification test, is a portion of each colony transferred with needle before the MF transfer or after the four-hour NA+ MUG incubation time?				Salar Sa	
Alternatively, is the membrane filter surface swabbed with a sterile cotton swab after the four-hour incubation time on NA + MUG and then transferred to a total coliform verification test?			TO THE STATE OF	The state of the s	
Is the inoculated NA + MUG medium incubated at 35±0.50 for four hours?	5.43.4		The state of		
Is fluorescence checked by using a UV lamp (365-366 nm) with a six-watt bulb in a darkened room and any fluorescence in the halo around a sheen colony considered positive for <i>E. coli</i> ?	5435				
MF method for detecting enterococci/fecal streptococci in ground water	5:4.4				
Media	5.4.4.1				

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Element	Number	Yes	No	NA	Comments
When mE agar is used for the detection of enterococcisis basal mE agar prepared, autoclaved, and cooled before the addition of nalidixic acid (or its sodium salt) and triphenyl tetrazolium chloride, both of which are added separately to the medium and mixed?	544.11				
Is the final pH of mE agar 7:1±0!2?  When m-Enterococcus agar is used for the detection of fecal streptococci (not enterococci), is the medium heated; not autoclaved; to dissolve the ingredients?	5.44.1.2				2 2 2
Is the final pH of m-Enterococcus agar 7.2±0.2?  When mEI agar is used for the detection of a enterococci, is 0.75g indoxyl-β-D-glucoside added to	5.4.4.1.3				
IE basal mE agar and then prepared according to 5.4.4.1.1 except that only 0.02 g/E triphenyl tetrazolium chloride is added?					
Is the final pH of mEI agar 7.1±0.2?  Is a 100-mL sample filtered and the membrane placed on one of the agar media previously listed?  If m-Enterococcus agar is used, are the plates.	5.4.4.2 5.4.4.3				254
incubated in an inverted position at 35±0.5°C for 48, hours?  Using magnification and a fluorescent lamp, are all					
light and dark red colonies counted as fecal streptococci?  If mE agar is used; are the plates incubated in an inverted position for 48 hours at 41°±0.5°C?	5.4.4.4				
Is the membrane filter then transferred to EIA medium and incubated at 41°±0.5°C for 20-30 minutes?  Using magnification and a fluorescent lamp, are all					COMPANY OF THE PROPERTY OF THE
pink to red colonies on mE agar with a black or reddish brown precipitate on the underside of the filter on EIA agar counted as enterococci?					

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Element	Number	Yes	No	NA	Comments	
If mEl agar is used, are plates incubated in an inverted position for 24 hours at 41±0.5℃?	5.4.4.5					
Using magnification and a fluorescent lamp, is the plate examined, top and bottom, for colonies with a blue halo (regardless of colony color) considered as positive for enterococci?						
Heterotrophic Plate Count (for enumerating heterotrophic bacteria in drinking water)	5.5					
Does the laboratory use the Pour Plate Method or/the SimPlate Method for enumerating heterotrophic bacteria in drinking water and for testing reagent grade water?	5.5.1	XV			Ex. 5 - Deli	berative
For systems granted a variance from the TCR's maximum contaminant level, does the laboratory use R2A medium with a method in <i>Standard Methods</i> , Section 9215 for enumerating heterotrophic bacteria in drinking water?				X		
Media	5.5.2					
Is the final pH recorded for plate count agar pH 7.0±0.2, R2A agar 7.2±0.2, and SimPlate 7.2±0.2?		X		ur.		1
For the Pour Plate Method, is melted agar tempered at 44-46°C in a water bath and maintained no more than 3 hours before pouring?	5.5.3	X	`			Ex. 5 - Deliberati
Is this sterile medium melted only once?		X				
For the Spread Plate Method, is 15 mL of R2A medium (or other medium) poured into a sterile petri dish and allowed to solidify?	5.5.4			x ./		
Is refrigerated medium in bottles or screw-capped tubes stored for no longer than six months, or in petri dishes for no longer than 2 weeks (one week for prepared petri dishes with R2A medium)?	5.5.5			X /		

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Element	Number	Yes	No	NA	Comments
For countable plates of most potable water samples, are 1.0 mL and/or 0.1 mL volumes of the undiluted sample plated?	5.5.6	X			
Are at least duplicate plates prepared per dilution tested?	,	X_			
For the Pour Plate Method, is the sample pipetted aseptically onto the bottom of a sterile petri dish and then at least 10-12 mL tempered melted agar added?	5.5.7	x V			
Is the sample and melted agar mixed, avoiding spillage?		X V			
After the agar plates have solidified on a level surface, are they inverted and incubated at $35\pm0.5$ °C for $48\pm3$ hours?		X.			
Are plates stacked no more than four high and arranged in the incubator to allow proper air circulation and to maintain a uniform incubation temperature?		×X			
Does the laboratory ensure that incubator does not have excess humidity and that the plates do not lose more than 15% by weight during the 48 hours of incubation?		X /			
For the Spread Plate Method, is 0.1 or 0.5 mL of the sample (or dilution) pipetted onto the surface of the predried agar in the plate and then spread over the entire surface using a sterile bent glass rod?	5.5.8			X	
Is the inoculum absorbed completely before incubating?				χ,	
Are the plates incubated in an inverted position at 20-28°C for 5-7 days?				X A	/
For the Membrane Filter Technique, does the filtered volume yield between 20-200 colonies?	5.5.9			X	/
Is the filter transferred to a petri dish containing 5 mL solidified R2A medium and then incubated at 20-28°C for 5-7 days?				x/	

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Element	Number	Yes	No	NA	Comments
Are plates with loose-fitting lids placed in a plastic box with a close-fitting lid and moistened paper towels, and rewetted as necessary?	;	Х		X	/
Are colonies counted using a stereoscopic microscope at 10-15X magnification?		X		X	
SimPlate Method	5:5:10:	# 		χV	
For a single sample Unit Dose; is a 10-mL test sample added to a test tube containing dehydrated SimPlate medium and then poured onto the center of a plate containing 84 small wells?	5:5:10:1				
Alternatively is 9-mL of sterile diluent added to the test tube containing the dehydrated medium, followed by a 1-mL sample, and the medium plus sample then poured onto the center of a plate containing 84 small wells?					
Is this mixture distributed evenly to the 84 wells and is the excess liquid drained into the absorbent pad on the plate?					
Is the plate inverted and incubated at 35±0.5°C for 45-72 hours?					
Is bacterial density determined by counting the number of wells that fluoresce under a 365=366-nm UV light; and converting this value to a Most Probable.  Number/mL using the manufacturer: S Unit Dose MPN table?					
If a 10-mL sample is used, is the Unit Dose MPN/mL a read directly or, if a 11-mL sample is used, is the MPN/mL value corrected by multiplying it by 10?					
For the Multiple Dose for 10 samples of 1 mL each is a 100-mL sterile diluent added to the dehydrated SimPlate medium and shaken to dissolve?	5.5:10:2				
Is a 1.0-mL test sample then pipetted to the center of a plate, followed by 9 mL of the reconstituted medium?					

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Element	Number	Yes	No	NA	Comments
Is the plate then gently swirled to mix and distribute the sample and medium mixture evenly to the 84 wells, with the excess liquid then being drained into the absorbent pad on the plate?					
Is the plate inverted and incubated at 35±0.5°C for 45-72 hours?					
Is bacterial density determined by counting the number of wells that fluoresce under a 365-366 nm UV light; and converting this value to a Most Probable Number/mL using the manufacturer's Multi-Dose MPN table?					
If sample dilutions were made during sample preparation; is the MPN/mL value multiplied by the dilution factor?					
For the Pour Plate and Spread Plate Techniques, are colonies counted manually using a dark field colony counter?	5.5.11	X i/			
Are only plates having 30 to 300 colonies counted, except for plates inoculated with 1.0 mL of undiluted sample where counts of less than 30 are acceptable?		X V			
QC Is each batch or flask of agar checked for sterility by pouring a final control plate?	5.5.12	X.			
QC Does the laboratory reject data if the control is contaminated?		X V			·
Coliphage (Draft Method 1601 and 1602 for the GWR)	5.6			x V	
EPA Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment	5.6.1				Ex. 5 - Delibera
Is the 100-mL (or 1-L) water sample supplemented with magnesium chloride log-phase host bacteria, and tryptic soy broth (TSB) as an enrichment step for coliphage?					V

Element Number		Yes	No	NA	Comments
After incubation overnight; are samples spotted onto a lawn of host bacteria, incubated, and then examined for circular lysis zones?		<b>经验验</b>			
Media	5.6.1.1				
For antibiotic stocks, are antibiotics always added to the medium after the medium has been autoclaved?	5:6.1.1.1		Tu .		
Are antibiotic stocks stored frozen at -20°C for no longer than one year?					
Are antibiotic stocks thawed at room temperature or rapidly in a water bath up to 3 TC and mixed well before using?					
Is 10X tryptic soy broth (TSB) stored at 1-5C until used?	5.6.1.1.2		机构数		
Are 1.5% tryptic soy agar (TSA) plates after antibiotic supplementation and solidification stored inverted at 1°-5°C for no longer than two weeks; if not used immediately?	5.6.1.13				
Are 0.7% TSA top agar tubes with appropriate antibiotics, dispensed as 5-mL per sterile 10-mL tube labeled, and kept at 45-48°C until used?	5.6.1.1.4				
Are these tubes used the day of preparation?					
For spot plates that develop condensation during storage are plates incubated for approximately 10 minutes to reduce condensation prior to inoculation?	5.6.1.1.5				
Are spot plates used that day or stored at 1°5°C for up to four days?					
Coliphage stock	5.6.1.2				
Are MS2 (ATTC#15597-B1 male-specific) and phi=X 174 stock coliphage (ATTC#13706-B1, somatic) stored at 2-8C for up to five years?	5.6.1.2.1				
Does analysis of raw sewage filtrate begin within 24 hours of collection?	5.6.1.2.2				

Element	Number	Yes	No	NA	Comments
Is at least 10 ml of filtered sewage obtained?	5.6.1.2.4		が高く		
If the filtrate is stored more than 24 hours, is it retitered before use?	5.6.1.2.5				
Host bacteria stock cultures	5.6.1.3				
After preparation, are host bacteria stock cultures held, at a temperature between -20°C and -70°C?	5.6:1.3.1				
Are bacteria stored no longer than two months at -20°C or no longer than one year at -70°C?					
Are prepared overnight host bacteria stock cultures: A chilled on wet ice or at 1°-5°C until ready to use?	5.6.1.3.2				
After preparation, are log-phase host bacteria stock cultures chilled on wet ice or at 1°-5°C to slow replication until ready for use?	5.6:1.3.3				
When stored are these suspensions stored no more than 48 hours?					
Are remaining bacterial host cultures stored at left overnight to inoculate flasks for the preparation of new log-phase bacterial hosts?					
General QC	5:6:1,4				
Initial demonstration of capability (IDC)	5:6.1.4.1				<b>1</b> -14
QC Did the laboratory demonstrated ability to generate acceptable performance with this method by performing an IDC test before analyzing any field samples?					
QC Did the IDC test consist of ten reagent water samples spiked with enumerated sewage filtrate or equivalent at 1-2 PFU mL per sample for every sample for each coliphage type used, according to the IDC Table?					
QC Were these IDC tests accompanied by a method blank for each coliphage type used?					
Method Blanks	5.6:1.4.2				

Element	Number	Yes	No	NA	Comments
QC Is a method blank (reagent water sample containing no coliphage) analyzed to demonstrate freedom from contamination?					
For each coliphage type used, is a sterile reagent water sample prepared and analyzed using the same procedure used for analysis of field and QC samples?					
QC Is at least one method blank analyzed for every spot plate used for field samples?					
Positive Controls	5.6.1.4.3.			がなる	
QC Are positive controls analyzed to ensure that stock coliphage suspensions, host bacterial cultures, and growth media are performing properly?					
QC For each coliphage type used, is a 100 mL reagent water sample spiked with 20 PFU from sewage filtrate or 60 PFU from a pure coliphage stock culture?					
QC Is one positive control analyzed for every spot plate used for field samples?					
QC If multiple spot plates are inoculated with samples on the same day, is a single enriched positive control sample used to inoculate multiple spot plates that day?					
Matrix spikes (MS)	5.6.1.4.4				
QC Is method performance assessed by analyzing one set of MS samples for each coliphage type used when samples are first received from a new ground water source?					
QC For each coliphage type analyzed, are three field samples spiked with 1-2 PFU, with at least one out of the three MS samples being positive for each coliphage type?					
<b>QC</b> Is one set of MS samples analyzed on an ongoing basis after every 20 <sup>th</sup> field sample for each ground water source?					

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Element	Number	Yes	No	NA	Comments
QC: Are these MS samples collected at the same time as routine field samples?					
QC: Are these samples spiked in "bulk" at the concentrations indicated in the MS and @DC Sample. Spiking Table?					
Ongoing demonstration of capability (ODC)	5.6.1.4.5				
QC Does the laboratory demonstrate that the analytical system is in control on an ongoing basis through analysis of ODC samples?					
QC: Are three reagent water samples spiked with 1-2. PFU for each coliphage type used?					
QC Are ODC test samples analyzed exactly like field samples?					
QC: Is a minimum of one out of three ODC samples positive for each coliphage type used?					
QC Is one set of ODC samples analyzed after every 20 field and MS samples or one per week, whichever occurs more frequently?				A CONTRACTOR OF THE CONTRACTOR	
QC Are samples spiked in "bulk" at the concentrations indicated in the MS and ODC Sample Spiking Table?					
Performance studies	5.6.1.4.6		100 mg/m		
QC Does the laboratory periodically analyze external QC samples when available?					
QC Does the laboratory participate in available interlaboratory performance studies?					
QC Does the laboratory review results; correct unsatisfactory performance; and record corrective actions?					
EPA Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Layer Agar (SAL)-Procedure	5.6.2				The second of th

Element	Number	Yes	No	NA	Comments
Is the 100-mL ground water sample supplemented with magnesium chloride and host bacteria; and then added to 100-mL of double-strength molten tryptic soy agar containing the appropriate antibiotic?					
Is the sample thoroughly mixed and the total volume then poured into five to ten plates?					
After overnight-incubation; are circular lysis zones recorded as coliphage?					
Media	5.6:2.1				
For antibiotic stocks, are antibiotics always added to the medium after the medium has been autoclaved?					
Are antibiotic stocks stored frozen at -20°C for up to one year?					
Are antibiotic stocks thawed at room temperature or rapidly in a water bath up to 3 °C and then mixed well-before using?					
Is 10X TSB stored at 1°5°C until used?					
Are 1.5% TSA plates; after antibiotic supplementation and solidification; stored inverted at 1°5°C for no longer than two weeks, if not used immediately?					
Are 0.7% TSA top agar tubes with appropriate antibiotics, dispensed as 5-mL per sterile 10-mL tube, labeled, and kept at 45-480 until used?					
Are these tubes used the day they are prepared?				¥1,44	
Are spot plates that develop condensation during storage, incubated for approximately 10 minutes to reduce condensation prior to inoculation?					
Are these plates used that day or stored at 1°-5°C for up to four days?					
Is 2X TSA, with appropriate antibiotics; kept molten at 4548C in a water bath?	5.6.2.1.2				
Is this agar used only on the day of preparation?					

Element	Number	Yes	No	NA	Comments
Coliphage Stock	5.6.2.2				
Are MS2 (ATTC#15597-B1; male-specific) and phi-X 174 stock coliphage (ATTC#13706-B1; somatic) stored at 2-8C for up to five years?					
Does analysis of raw sewage filtrate begin within 24 hours of collection?					
Is at least 10 mL of filtered sewage obtained?					
If the filtrate is stored more than 24 hours, is it re- titered before use?					
Host bacteria stock cultures	5.6.2.3				
After preparation, are host bacteria stock cultures held at a temperature between -200 and -700?					
Are bacteria stored no longer than two months at -20°C; or no longer than one year at -70°C?					
After preparation, are overnight host bacteria stock cultures chilled on wet ice or at 1.50 until ready for use?					
After preparation, are log-phase host bacteria stock cultures chilled on wet ice or at 1-5°C to slow.					
When stored, are these suspensions stored no longer than 48 hours?					
Are remaining bacterial host cultures stored at 1250 overnight to inoculate flasks for the preparation of new log-phase bacterial hosts?					
General QC	5.6.2.4				
Initial precision and recovery (IPR)	5.6.2.4:1				
QC Has the laboratory demonstrated the ability to perform this method acceptably by performing an IPR test before analyzing any field samples?					

Element .	Number	Yes	No	NA	Comments
QC - Does the IPR Test consist of four reagent water samples tested for each coliphage type used, spiked with enumerated sewage filtrate or equivalent to yield a target spike concentration of 80 PFU per sample?					
QC Does the relative standard deviation of the recovery (RSD), and the average percent recovery (D) based on all four sample results for each coliphage type used meet the acceptance criteria in the QC Acceptance Criteria table?					
Method blanks	5.6:2.4:2				
QC Is one method blank (reagent water sample: containing no coliphage) analyzed with each analytical batch to demonstrate freedom from containination?					
QC. For each coliphage type used, are sterile reagent water samples prepared and analyzed using the same procedures used for analysis of the field and QC samples?					
QC Is an analytical batch defined as all samples analyzed during a single day, up to a maximum of 20 samples (field samples and matrix spike samples) per coliphage type used?					
Matrix spikes (MS)	5.6.2.4.3				
QC. Is method performance assessed by analyzing one set of MS samples for each coliphage type used when samples are first received from a new ground water source?					
QC is one set of MS samples routinely analyzed after every 20 <sup>th</sup> field sample for each ground water source?					
QC If the recovery for coliphage falls outside its limit (see the QC Acceptance Criteria table), is method performance considered unacceptable?					
QC If OPR results are not within control limits; is the problem identified and corrected and the data qualified?					

Element	Number	Yes	No	NA	Comments
QC Does the laboratory maintain a control chart on recovery and update it on a regular basis?					
Ongoing precision and recovery (OPR)	5.6.2.4.4				
QC Does the laboratory demonstrate acceptable performance through analysis of an OPR sample on an ongoing basis?					
QC For each coliphage type used; is a reagent water a sample spiked with approximately 80 PFU?				****	
QC-lis the OPR sample analyzed exactly like a field sample?					
QC Is one OPR sample analyzed for each analytical batch?					
QC#Does the laboratory compare the OPR percent recovery (R) with the corresponding limits in the QC Acceptance Criteria table?					
QC If R falls outside the range for recovery, is analysis stopped until the problem is identified corrected and another OPR test is successfully performed?					
Performance studies	5.6.2.4.5				
QC Does the laboratory periodically analyze an external QC sample when available?					
QC Does the laboratory participate in available interlaboratory performance studies?					
QC Does the laboratory review these results, correct unsatisfactory performance, and record corrective actions?					
6. SAMPLE COLLECTION, HANDLING, AND PRESERVA	TION	26812	50-89 <b>8</b>	SARPACIFI (	2.23.4.45.25.35.1.6.18.18.18.4
Sample Collector	6.1				<del></del>

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Element	Number	Yes	No	NA	Comments
Is the sample collector trained in aseptic sampling procedures and, if required, approved by the appropriate regulatory authority or its designated representative?		X			
Sampling	6.2				
Are the drinking water samples collected under the Total Coliform Rule representative of the water distribution system?	6.2.1	X			
Are the water taps used for sampling free of aerators, strainers, hose attachments, mixing type faucets, and purification devices?		X			
Are only cold water taps used?		Χν			
Are service lines cleared before sampling by maintaining a steady water flow for at least 2 minutes or until a steady water temperature is reached?		X			
Is at least a 100-mL sample volume collected, allowing at least a 1-inch air space in the container to facilitate mixing of the sample by shaking?		X			
Is a sample information form completed immediately after sample collection?		X	/		
If a sample bottle is filled too full to allow for proper mixing, is the entire sample poured into a larger sterile container and mixed before proceeding with the analysis?		x ✓		,	g.
For the SWTR, are the source water samples representative of the source of supply and collected not too far from the intake point, but at a reasonable distance from the bank or shore?	6.2.2	X 🗸			
Is the sample volume sufficient to perform all the tests required?		X V	and the second second	·	
For the analysis of coliphage, <i>E. coli</i> , or enterococci under the GWR, is at least a 100-mL sample volume collected?	6.2.3 6.2.4			X	

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Element	Number	Yes	No	NA	Comments	
Sample Icing	6.3	1/				
For drinking water bacterial samples, is the sampler encouraged to hold samples at <10°C during transit to the laboratory?	6.3.1	Y	X			
For source water bacterial samples, are samples held at <10°C during transit to the laboratory?			X		_ - Ex. 5 - Delib	orativo
Does the laboratory reject samples that have been frozen?		$\mathbf{x}^{V}$		?	EX. 3 - Della	erative
For coliphage analysis under the GWR, are samples shipped at <10°C, stored at 1°-5°C, and not frozen?	6.3.2	l V	<i>[</i>	х		
<b>QC</b> For SWTR samples and coliphage samples, does the laboratory record sample temperature upon receipt?	6.3.3	X				
QC Does the laboratory flag samples that have a temperature upon receipt of >10°C, whether iced or not, unless the time since the sample collection is less than two hours?		X				
Sample Holding/Travel Time	6.4					
For the analysis of total coliforms in drinking water, does the time between sample collection and placement of the sample in the incubator not exceed 30 hours?	6.4.1	X		÷		
Are all samples analyzed on the day of receipt?		Χį⁄				
Are samples received late in the day refrigerated overnight only if analysis can begin within 30 hours of collection?		-		X		
For total coliforms and fecal coliforms in surface water sources, and for heterotrophic bacteria in drinking water, is the time from sample collection to placement in the incubator less than eight hours?	6.4.2		X_		Ex. 5 - Deli	berative
For coliphage analysis, is the time from sample collection to placement of sample in the incubator less than 48 hours?	6.4.3			X		

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Element	Number	Yes	No	NA	Comments
For coliphage analysis, is the time from sewage sample collection to analysis of QC spiking suspension less than 24 hours, unless re-titered and the titer has not decreased by more than 50%?				X	
If the titer has not decreased by more than 50%, is the sample stored no longer than 72 hours?				x/	
For <i>E. coli</i> and enterococci analysis under the GWR, is the time between sample collection and the placement of sample in the incubator less than 30 hours?	6.4.4			X	<i>(</i> .
Sample Information Form	6.5	Χ.,			

laboratory? 7. QUALITY ASSURANCE				·
Are applicable State regulations pertaining to chain-of- custody followed by sample collectors and the		X		
Chain-of-Custody	6.6			
After collection, does the sampler enter the following information, in indelible ink, on sample information form?  Name of system (PWSS identification number if available) Sample identification (if any) Sample site location Sample type (e.g., a routine distribution, repeat, raw or process, or other special purpose) Date and time of collection Analysis requested Disinfectant residual Name of sampler Any remarks		X/		

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Does the laboratory have a written QA Plan prepared and available for inspection?	7.1	X .		
If yes and if this is separate from a chemistry QA Plan please copy and complete the ?? Section of the Chemistry Pre-survey plan entitled "VII.B.1".				JOINT QA MANUAL WITH CHEM
Does the laboratory follow the written QA Plan?		x v		
Does the laboratory have a Standard Operating Procedure available for review pertaining to its own calibration of equipment or supplies?		X <sub>V</sub>		
Does the laboratory successfully analyze at least one set of PT samples once every 12 months for each method for which it is certified?	7.2	X./		
For methods used to test the presence or absence of an organism in a sample, does the laboratory analyze each PT sample set using a single analytical method only?		X /		,
8. RECORDS AND DATA REPORTING				
Legal Defensibility	8.1		1	
Are compliance monitoring data being maintained by the laboratory both thorough and accurate, and thus legally defensible?		X/		
Does the laboratory's QA plan and/or SOPs describe the policies and procedures used by the facility for record retention and storage?		X		
If samples are expected to become part of legal action, does the laboratory follow chain-of-custody procedures?		x		
Maintenance of Records	8.2			
Does the public water system maintain records of microbiological analyses for five years?	-	?		
Does the laboratory maintain easily accessible records for five years or until the next certification data audit is completed, whichever is longer?		X,		

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Does the laboratory notify the client water system before disposing of records so they may request copies if needed?		X		
Does the laboratory backup all electronic data by protected tape, disk, or hard copy?		X		
When the laboratory changes its computer hardware or software, are provisions in place for transferring old data to the new system so that data remain retrievable within the specified time frames?		X		
Sampling Records	8.3			
Are all data recorded in ink, with any changes lined through such that the original entry is visible?		×		
Are changes initialed and dated?		Χį		
Does the laboratory have the following sample information readily available?  Date and time of sample receipt by the laboratory Name of the laboratory person receiving the sample  Information on any deficiency in the condition of the sample	8.3.1-4	X		

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i	 i	 	
Are samples invalidated for the following reasons?  Time between sample collection and receipt by laboratory exceeded  Presence of disinfectant in sample noticed, e.g., odor  Evidence of freezing  Use of a container not approved by the laboratory for the purpose intended  Insufficient sample volume, e.g., <100 mL  Presence of interfering contaminants noticed, e.g.	X		
hydrocarbons, cleansers, heavy metals, etc. Sample temperature exceeding the maximum allowable		:	

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Analytical Records	8.4						
Are all recorded data in ink with any changes lined through such that original entry is visible?		X	Z				
Are these changes initialed and dated?	,	X /	,				
Are the following readily available?  Laboratory sample identification information Information concerning date and time analysis begins Name of the laboratory and a signature or initials of the person(s) performing analysis Information concerning the analytical technique or method used Information concerning all items marked "QC" Results of the analyses	8.4.1-6	x	<i>'</i>				
Preventive Maintenance	8.5			Fv	5 -	Dali	berati <sup>,</sup>
Does the laboratory maintain preventive maintenance and repair records for all instruments and equipment?		X	,		. 0 -	Deli	Derati
Are these records kept for five years in a manner that allows for easy inspection?		X /	\				
9. ACTION RESPONSE TO LABORATORY RESULTS							
Testing Total Coliform-Positive Cultures	9.1						
For the Total Coliform Rule, does the laboratory test all total coliform-positive cultures for the presence of either fecal coliforms or <i>E. coli</i> ?		x /					:
Notification of Positive Results	9.2						
For Total Coliform Rule, does the laboratory promptly notify the proper authority of a positive total coliform, fecal coliform, or <i>E. coli</i> result, so that appropriate follow-up actions can be conducted?	9.2.1						
For the Total Coliform Rule, if a sample is fecal coliform- or <i>E. coli</i> -positive, does the system notify the State as soon as it is notified of the test result, i.e., at the end of that day or, if the State office is closed, by the end of the next business day?	9.2.2	х _/			. /		

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Does the laboratory base a total coliform-positive result on the confirmed phase if the Multiple Tube Fermentation Technique or Presence-Absence Coliform Test is used, or the verified test for the Membrane Filtration Technique if M-Endo medium or M-Endo LES agar is used?	9.2.3	X V		
If a presumptive total coliform-positive culture does not confirm/verify as such, but is found to be fecal coliform or <i>E. coli</i> -positive, is the sample considered total coliform-positive and fecal coliform/ <i>E. coli</i> -positive?		***************************************	,	
Notification of Total Coliform Interference	9.3			
For the Total Coliform Rule, does the laboratory promptly notify the proper authority when results indicate non-coliforms may have interfered with total coliform analysis?		X.		























# Ex. 5 - Deliberative

#### **DIRECTIONS FOR BACTERIOLOGICAL SAMPLING**

#### **COLLECTING THE SAMPLE**

- Use only sterile sample bottle turnished by State or County Health Departments. These sample bottles have a six month shelf life after which they must be returned to the Office of Laboratory Services for
- Do not touch the inside of the sample bottle or cap or otherwise contaminate outfit.
- Do not collect from a storage tank, leaky faucet, aerators, or "purifiers".
- Allow water to run for 5 minutes to clean service line before sampling.

  Do not overflow or rinse sample bottle.

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- Fill sample bottle to the shoulder leaving about a 1 inch air space at the top. 6.
- Replace the sample bottle cap securely.

#### COMPLETING THE SAMPLE HISTORY - REPORT FORM

- Complete all of the following information IN INK make sure that all copies are legible. 1.
- Provide the following information:
  - County of water sample origin.
  - Public Supply (PWS) ID Number and name of water supply.
  - Who is to be charged for the sample examination?
  - Đ. Collector's name, title, certification number, organization, and telephone number
  - To whom the final report of examination is to be mailed? (DO NOT WRITE "SAME AS E.
  - ABOVE" This information appears in a window envelope.)
  - Bottle Number.
- Complete the following sample collection data:
  - Sample Type Repeat Samples and Réplacement Samples must have the complete lab number of the previous sample that they are a Repeat/Replacement for. (Repeat samples are for samples that were previously Total Coliform Positive and must include their source: Original Location, Upstream, Downstream or Other; Replacement Samples are for samples that were previously Not Reported: Unsatisfactory, Laboratory Accident or Invalid.)
  - Date and Time of sample collection. COLLECTOR MUST INITIAL THE FORM.
  - Give a specific description of the Sampling Point.
  - Is the Water Supply Chlorinated? Chlorine Residual. Ex. 5 Deliberative

  - How the sample is to be transported to the laboratory and the transportation condition.

#### MAILING - DELIVERY TO LABORATORY

- Samples must be sent or brought for receipt to the laboratory in time for examination during the following hours (South Charleston Laboratory: 8:00 am to 4:30 pm, Monday thru Friday. Kearneysville Laboratory: 8:00 am to 4:00 pm Monday thru Wednesday and 8:00 am to 12:00 pm, Thursday) and within 30 hours after collection.
- 2. Check departure schedule of mail or delivery service from your area and plan for collections to be readied for shipment at that time.
- Make sure postage is affixed to outer mailer.

ALL FIVE COPIES OF THE COMPLETED HISTORY FORM MUST BE ENCLOSED WITH THE SAMPLE.

SAMPLING CONTAINERS ARE THE PROPERTIES OF THE STATE AND THEIR USE IS RESTRICTED ONLY FOR THE COLLECTIONS BY STATE AGENCIES OR THOSE DULY AUTHORIZED BY THE STATE.

MICROBIOLOGICAL ANALYSIS RECORDS ARE DISPOSED OF AFTER 5 YEARS.

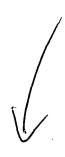
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### BENCH SHEET- MTF

Lab Sample #	Code	Collect Date	Analy Date	e County	Name/Co	Collector	Sample Loc	P/A2	P/A4	Initials/Date/Time	Conf24	Conf48		Total	Feca	RDat	e initial	sRead Time
85590	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	1.		+	mike 2-3-10	+1	= tmi	-1	Pro	Abs	a-4	اساكم	93AFF
85591	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	. 2	<del>- (</del>	-1	Ar 2-3 70 2 7 12	-	·				2-3	سناك	Gna
85592	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	3	-1	T	m= 2-3-10	-1-	370	-1			2-5	nite	93.40
85593	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	4	-1	-1			7						din
85594	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	5		+1	MF 2-3-10	+1		+	Pies	المحا	<b>5</b> .4	مه	97,54
85595	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	6 .		+1	ME 2-3-10	+ 1							C/50 1/4 @
85596	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	7		+1	m=2-3-10	+1		-1	Pies	Abs	2-4	Me	43500
85597	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	8		+1	MF 2-3-10	+1							c130,
85598	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	9		+1	m= 2-3-10	+1		-1	Pies	<da< td=""><td><b>2-4</b></td><td>ماله</td><td>Bertin</td></da<>	<b>2-4</b>	ماله	Bertin
85599	20	1/31/2010	2/1/2010	KANAWHA	WS 162 MULTI TUBE	ND	10	-1	-1	1						2-3	Mye	9Am

Monday, February 01, 2010

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Current QM

# ENVIRONMENTAL MICROBIOLOGY WATER SAMPLE COLLECTION AND HANDLING

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## Ex. 5 - Deliberative

ENVIRONMENTAL MICROBIOLOGY WATER SAMPLE COLLECTION & HANDLING

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#### 1. Introduction

Sample Handling is a critical aspect of the examination process. Without maintaining sample integrity, test results are meaningless. This section deals with all aspects of sample handling for the Water Program. Discussions will be included on Sampler Training, Sample Scheduling, Sample Collection, Transportation, Sample Accession, Storage and Disposal.

#### 2. Training for Samplers

- 2.1. Water samples are received from water plant operators, district engineers, county and state sanitarians, contracting firms, business owners and private individuals. To submit samples for Compliance (compliance with the Safe Drinking Water Act), and individual must have at minimum a Class 1-D Operators License. Training is provided in the following manner:
  - Water Plant Operators Receive training at the Water Plant Operators Course held at the Environmental Training Center in Ripley, WV.
  - District Engineers Receive on-the-job training.
  - County and State Sanitarians Receive training at the Sanitarian Training Course held at the Office of Environmental Health Services. They also receive on-the-job training.
  - Business Owners People that own establishments that have their own wells that serve
    the public must receive training from the Office of Environmental Health Services,
    Environmental Engineering Division.
  - Contracting Firms and Private individuals Receive no formal training but are provided detailed instructions on the back of the Water Bacteriological Report Form.
- 3. <u>Sample Scheduling:</u> Water samples for compliance purposes are submitted based on schedule setup by the Office of Environmental Health Services Environmental Engineering Division. Other types of water samples are not scheduled. Clients are discouraged from submitting samples on Weekends.

#### 4. Sample Collection

- 4.1. Water samples are to be collected only in vessels supplied by the Office of Laboratory Services. There are two types of collection vessels used a 4 oz. nalgene bottle that is laboratory processed and reused and clear, disposable vessels provided by IDEXX. Only by special permission of the section supervisor may another type of bottle be used.
- 4.2. Collection vessels are mailed out to clients of the Office of Laboratory Services by the Container Section. Collection vessels may also be picked up in person by stopping by the laboratory.
- 4.3. Sample collection must be performed as described in Attachment #2
- 4.4. After sample collection, the Water Bacteriological Report Form (EM-1) Attachment #3 is to be completed as described in Attachment #4.

#### 5. Sample Accession

5.1. 90% of samples are picked up from the South Charleston Post Office by maintenance personnel and delivered to the General Reporting Office where the samples are sorted according to the appropriate laboratory sections. Samples that are shipped to the laboratory by other couriers (UPS, FedEx or DHL) are delivered to the Fiscal and Inventory Management Section and then delivered to Environmental Microbiology. Samples are also brought in to the

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- laboratory by clients and left at the front desk throughout the day. The receptionist notifies the section each time samples are left at the front desk.
- 5.2. Upon receipt by the Environmental Microbiology Section the samples are sorted according to Test Method and Sample Codes. See Attachment #10 for a list of Test Methods and Sample Codes and Attachment #11 Test Method Chart. Sample vessels are set on top of the water bacteriological report form (they must be kept together). A three digit number sticker is placed on top of the sample vessel (the last three digits of the 5 digit laboratory number) and the water bacteriological report form is stamped with the laboratory number and date received. The water bacteriological report from is then marked with the test method, time received, initials of analysts receiving samples, analysis date and time and initials of analysts performing the analysis.
- 5.3. Water bacteriological report forms are then entered into the computer using Microsoft Access.

  The following fields are entered:
  - Lab Number
  - Test Method and Sample Code
  - County of Origin
  - Date of Collection, Receipt and Analysis
  - Supply
  - Mailing Address
  - Collector
  - Public Water Supply ID Number
  - Sampling Point
    - Compliance, Special Purpose or Repeat
- 5.4. The data base is used for printing the daily worksheets, locating samples for phone inquiries and compiling monthly reports.
- 5.5. Water samples will not be analyzed for any of the following reasons:
  - Exceeded Time (30 hours for compliance with the SDWA and samples requiring counts, 48 hours for all others)
  - Sample Contains < 100 mL
  - Insufficient Information (No date and time of collection or no phone number)
  - Sample contains residual chlorine
  - Insufficient air space to facilitate mixing
  - Unauthorized Collector
  - For any samples not analyzed, a replacement sample is requested.
- 6. <u>Sample Storage</u>: Water samples are analyzed immediately upon sample accession unless they are received at 4:30 pm. Samples received at 4:30 pm are stored in a wire basket in the sliding door refrigerator located in the Milk Room at 0.0 4.4°C (as long as the holding times will not be exceeded); unless the holding time will expire by the next morning or results are needed the next day as in the case of a "Boiled Water Advisory", then those sample will be analyzed up until 4:30 pm using Colilert 18. All samples analyzed after 1:30 pm are by Colilert 18.

#### 7. Sample Disposal

7.1. Excess water from water samples (sample remaining after use of 100 mL for analysis) is collected in wax buckets and disposed of down the sink unless sewage is suspected in which

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- case the remaining sample is left in the vessel and is taken to the Media/Glassware Section for autoclaving .
- 7.2. All multi tube fermentation tests (100 mL, 10 tube and dilutions), are taken to the Media/Glassware Section for autoclaving and reprocessing.
- 7.3. Negative Colilert 100 mL samples are poured down the sink and the vessels disposed of in the hard trash.
- 7.4. Positive Colilert 100 mL samples have > 2mL of bleach added to them, mixed, and left overnight, then poured down the sink and the vessels disposed of in the hard trash.
- 7.5. Quanti Trays are placed into autoclave bags and taken to the back autoclave for disposal.
- 7.6. HPC plates are placed into autoclave bags and taken to the back autoclave for disposal.
- 7.7. Nalgene sample vessels are taken to the Media/Glassware section for washing, autoclaving and reprocessing.

#### **MICROBIOLOGY SAMPLE FORM**

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#### **ENVIRONMENTAL MICROBIOLOGY**

Rocco S. Fucillo Cabinet Secretary

November 16, 2012

David E. Russell, Ph.D.
U.S. Environmental Protection Agency, Region III
Office of Analytical Services and Quality Assurance
701 Mapes Road
Fort Meade, MD 20755-5350

Dear Dr. Russell.

The following is a list of corrective actions along with supporting documentation that have taken place since the September 18-19, 2012 triennial on-site evaluation at the Office of Laboratory Service's Drinking Water Laboratory:

1. Paragraph 8.4 (Chapter V, CLADW), lists the essential items to be captured in all analytical records. These include the result of the analysis. Currently, when performing presence/absence drinking water methods, the Laboratory only records on the bench sheet a positive (present) result for total coliforms. Fields left blank indicate negative (absent) results for total coliforms. Although it is understood within the Laboratory what the blank field indicates, this practice does not conform with paragraph 8.4 or with general conventional practice in microbiology. A result must be recorded for every test. SOPs need to be updated to reflect this revised procedure. (It should be noted that both positive and negative results are recorded on the bench sheet for E. coli.)

SOPEMW-002-R3.0; Sect 11.2. Pg 38-43

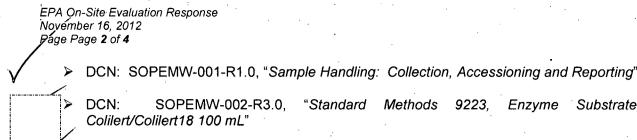
Response: Absents are now recorded in the bench sheets. The SOP's reflect this. See the Attached SOPs: SOPEMW-002-R3.0, "Standard Methods 9223, Enzyme Substrate, Colilert/Colilert18 100 mL" and SOPEMW-003-R2.0, "Standard Methods 9223 QT, Enzyme Substrate, Colilert/Colilert18 Quanti Tray".

2. Paragraph 11.3 (Chapter III, CLADW), as revised by Region III, states requirements for the management of laboratory SOPs. Although the Laboratory has in place a recently updated and signed SOP for Colilert (Presence/Absence) (Rev. 2.0 – 08/2012), SOPs for other methods are substantially out of date having not been revised or updated for several years. These include Colilert Quanti-Tray (2006), Multiple Tube Fermentation (2000), and Heterotrophic Plate Count (1998). Updated versions of these SOPs with signature pages need to be prepared.

Response: All SOPs are undergoing a major revision. The following SOPs have been completed, signed and included as attachments to this response:

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Phone: (304) 558-3530 FAX: (304) 558-2006



DCN: SOPEMW-003-R2.0. "Standard Methods 9223 QT. Enzyme Substrate, Colilert/Colilert18 Quanti Tray"

Methods

9223.

Enzvme

Substrate.

DCN: SOPEMS-007-R1.0, "Ethics and Data Integrity"

DCN: SOPEMW-004-R2.0, "Multi Tube Fermentation 100 mL & 10 x 10 mL"; DCN: SOPEMW-005-R1.0, "Heterotrophic Plate Count" and DCN: SOPEMW-006-R1.0, "Quality Control - Water Program are currently in the works and will be submitted as they are completed and signed.

"Standard

Findings concerning aspects of the drinking water microbiology quality system reported n the Inorganic Chemistry and Laboratory Quality System Report (2012) are hereby referenced and incorporated into this report. Addressed in Quality System Report

Response: The entire Quality System of the Drinking Water Microbiology Program is being "over-hauled". The findings listed in the Inorganic Chemistry and Laboratory Quality System Report are addressed in a separate correspondence.

#### F. Recommendations:

The following remarks are offered as suggestions to help improve the quality and integrity of the data the Laboratory generates.

It is requested that the laboratory provide feedback ("pros" and "cons") on the following suggestions offered in the spirit of continuous improvement:

Both paragraph 6.3.1 and the Federal Register (40 CFR 141.21(f)(3) footnote 2), in regard to the collection of drinking water samples from distribution systems, state, "Systems are encouraged but not required to hold samples below 10°C during transit." Accordingly, it is recommended that distribution system samples be held below 10°C during transit and that this condition be documented through the use of a temperature blank, the temperature of which would be determined upon arrival at the Laboratory and recorded.

Response: This would require the purchase of special shipping containers and freeze packs to accommodate hundreds of samples. It would also dramatically increase operating costs for both the laboratory and public water supplies by increasing the postage for the added size and weight of the new containers. At this time, it is not fiscally practical to accomplish this.

According to paragraph 3.15.4, "sufficient sodium thiosulfate must be added to a sample 2. bottle before sterilization to neutralize any residual chlorine in the water sample." Laboratory currently uses sample bottles containing sodium thiosulfate in powder form. The Laboratory should consider ways to confirm that the sodium thiosulfate contained in sample bottles is "sufficient" to neutralize the residual chlorine present in the water sample. One suggestion is to routinely check (using a check box on the Water Bacteriological Report) that the residual chlorine recorded on the report form by the sample collector is a residual concentration that can be neutralized by the amount of sodium thiosulfate known to be in the sample bottle. According to IDEXX the pre-sterilized containers with sodium thiosulfate contain 10-35mg of sodium thiosulfate which will neutralize a minimum of 10 ppm residual chlorine. Laboratory staff could simply check the report form to be sure the residual chlorine recorded is less than or equal to 10 ppm and then check a box on the form documenting that this procedure had been completed. Alternatively, it is suggested the Laboratory use commercially available potassium iodide strips to test a small aliquot of the sample, transferred to a watch glass, for the presence of residual chlorine. If present the strip will change color indicating that the sodium thiosulfate had not been sufficient to neutralize all the residual chlorine in the water sample. If the sodium thiosulfate had been sufficient, no residual chlorine would remain and the color change would not occur. Whatever means of performing this check is decided upon, it should be described in method SOPs or in the Laboratory's Quality Assurance Manual.

**Response:** Although this is a recommendation under the microbiology report, it appears to be a requirement under the Quality Systems Report, Item 1.:

- "1. The procedures used for <u>sample receiving</u> need to be documented for environmental chemistry and microbiology [Region III "musts" for CLADW, III-5, 11.1, 11.4 &11.5]. This needs to include:
  - · recording information in the Sample Log-in book and the Star LIMS,
  - the numbering system for samples including the designation of "regulated" and "COC", etc.;
  - checking and recording of necessary preservation checks; and

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• who is responsible (position) for these tasks.

We have implemented using Potassium Iodide Strips and documenting the results. The procedure is outlined in each method SOP. See Attached.

3. The Laboratory's Water Bacteriological Report (WBR) form captures a substantial amount of essential information, and the Laboratory is hereby commended for steadily improving it through a number of revisions. When next revised, or even prior to the next revision (because space on the form is currently available directly under "Laboratory Results"), it is recommended that the Laboratory include the date and time results are read on the form. This information is currently documented on the laboratory bench sheet, however, it is the only critical piece of information missing from the WBR. All other key dates and times are currently recorded on the WBR. Addition of the date and time results are read would make the WBR a complete record of sample collection, receipt and analysis. Currently neither the bench sheet or the WBR captures everything.

**Response:** Water Bacteriological Report Forms are currently being printed. This change was not included on the revision that is currently being printed; however, it may be considered for future revisions.

4. A further improvement of the Water Bacteriological Report would be to include the units of the chlorine residual recorded by the sample collector. This should be added to the training provided sample collectors with an explanation of why determining and recording the chlorine

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EPA On-Site Evaluation Response November 16, 2012 Page Page **4** of **4** 

residual is so important (so that the lab can evaluate the sufficiency of the amount of sodium thiosulfate added to the sample bottles prior to sample collection).

**Response:** Water Bacteriological Report Forms are currently being printed. This change was included on the revision that is currently being printed. A sample of the changes being made is demonstrated in Appendix B of DCN: SOPEMW-001-R1.0, "Sample Handling: Collection, Accessioning and Reporting"

5. Although a few years ago the Laboratory significantly improved its record of thermometer calibration by devoting one page of the log book to each thermometer--allowing anyone to easily review the history of calibration of any one thermometer--the current record system has moved a bit away from that simple system. Currently, one page is devoted to each thermometer when it is in a particular location. So a single thermometer may have several pages, each a different location. It is recommended each thermometer have one page devoted to its calibration and that the location of the thermometer simply be a field on the line documenting a thermometers annual calibration.

**Response:** This will be incorporated in the new Water Room Quality Control Book. The plan is to further separate the common items from the Water Program and the Dairy Program such as Balances, Pippettes, Thermometers, etc.

The staff, administration and I would like to thank you for the positive evaluation report. All staff, myself included, learned from this experience and are constantly seeking to improve and streamline procedures and techniques. If you need additional information, please don't hesitate to ask.

Sincerely,

Thomas L. Ong, HHR Program Manager I Chief – Laboratory Certification Officer (Micro)

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## Ex. 5 - Deliberative

## IBT Bulletin

2931 Moose Trail, Elkhart, IN 46514 1-800-490-5500 Fax: 574-264-2787

## The Most Commonly Misused Test Strips in Dialysis

Many reagent test strips use a color indicator which will react to a variety of different analytes. One example is the strips for peroxide and chlorine testing, both of which use reduction/oxidation (redox) indicator. As a consequence, many of these strips are improperly used to test for chemicals not intended for the specific application of the strip. The following are some of the most commonly misused test strips in renal dialysis community.

#### Hemastix

Hemastix is a reagent strip for detection of blood in urine. It is manufactured by Bayer Diagnostics in Elkhart, Indiana. There are other urinary blood test strips by other manufacturers under different trade names.

Hemastix essentially is a peroxide test strip. Its active ingredients include a peroxide substrate and a redox indicator. In the presence of blood, the blood component hemoglobin, causes the peroxide to reactwith the indicator and turn the strip blue. Since peroxide inherently could react with the indicator without hemoglobin, the strip is deliberately formulated to reduce the strip sensitivity for peroxide to prevent premature greening of the strip. As a consequence, the strip is also less sensitive to other oxidants, such as chlorine. When the strip is used for chloring testing, its detection sensitivity is about 5 ppm. Which means that when the strip is tested negative, the rinse water may still contain as much as -5 ppm of chlorine, which is 10 times above the recommended maximum allowable level of 0.5 ppm by the Association for the Advancement of Medical Instrumentation (AAMI).

WaterCheck RC, WaterCheck CP and WaterCheck 2 reagent strips from IBT will detect chlorine of less than 0.5 ppm down to 0.1 ppm or less. They are specially developed for use in dialysis settings.

#### Potassium Iodide Strip

Starch iodide strip is another example of the most commonly cross used reagent strips for oxidants. The strip is widely used for detection of peroxide or chlorine/bleach.

The strip contains potassium iodide as an active

ingredient. In the presence of an oxidant such as peroxide or <u>chlorine</u>, iodide is converted to iodine which then binds to starch molecules in the strip and forms a blue colored complex. The color intensity of the blue starch complex varies widely, depending on the source and the type of starch used in the strip formulation. Molecule for molecule, it is much less intense than other more complex redox indicators. The detection sensitivity of the strip for peroxide or chlorine is poor. They are primarily used only as potency test strips for bleach sanitizing solutions in food and other chemical industries.

When used for residual chlorine testing, the sensitivity of the starch/iodide paper is about 7 ppm which again is more than 10 times over the AAMI standard of 0.5 ppm or less.

Although the strip is used for testing of bleach potency in sanitizing solutions, it is not suitable for use as potency test for bleach disinfection solution. For disinfection, the chlorine level of the bleach solution is much higher than that in a sanitizing solution. In a bleach disinfection solution, the chlorine levels range from 500 to 5000 ppm, while in a sanitizing solution, the chlorine levels are usually around 100 to 300 ppm. At 100 ppm, the starch iodide paper will turn to dark blue/black and lose the capability to distinguish chlorine levels of 100 and 500 ppm, let alone 5000 ppm. When used as a potency test for the presence of bleach in the disinfection solution, the starch-iodide paper can not sufficiently assure a minimum of 500 ppm chlorine in the disinfection solution.

WaterCheck RC and WaterCheck CP offers a wide range of chlorine detection. The strips are capable of detecting chlorine levels of 500 ppm or higher and can be used as a potency test for the presence of chlorine disinfection solution.

### AquaCheck 5

AquaCheck 5 is a monitoring test strip, primarily, for swimming pool water. It contains 5 reagent pads in one strip, including tests for free and total chlorine, pH, hardness and alkalinity. The free and total chlorine pads are potency tests to assure certain minimum level of free chlorine in the water. Since both tests are potency tests, they do not have sufficient sensitivity to detect chlorine at 0.1 ppm levels. Nor is it possible to calculate and assure a concentration of 0.1 ppm or less of chloramine from the free and total chlorine as required in the dialysis applications.

## Use of Residual Test as a Potency Test

One other common misuse of the strip is to use a residual test strip for monitoring of high level disinfectant potency. Most residual reagent strips have a narrow sensitivity range, for example, at 5 ppm chlorine. The strip is not capable of discerning chlorine concentrations above that level. For disinfection, the chlorine concentrations are in the range of 500 to 5000 ppm. Testing merely for presence of chlorine provides no indication as to the potency and usefulness of the disinfecting solution.

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Integrated Biomedical Technology, Inc. (IBT) 2931 Moose Trail, Elkhart, IN 46514 USA

Phone: 1-800-490-5500 Fax: 574-264-0025

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- · Accurate for a wide range of routine pH testing
- Inexpensive
- Convenient and portable for field use

## pH Indicators

Strips Type CF

Individual plastic support strips carry four different segments of dye-impregnated indicator papers. The resulting combination of color differences gives an extremely clear and accurate visual pH value. All the dyes are chemically bonded to the paper and cannot be leached into solution; problems associated with contamination of the sample and resultant anomalous readings are avoided.

Strips Type CS

Each test strip has a central segment of indicator dye and, printed alongside, 8 or more different color segments marked with corresponding pH values for matching purposes. The pH test value can be read off by direct comparison of the test strip color and the color bars. Ideal for colored solutions, when any changes in color of the paper stock are automatically cancelled out.

Dispensers Type TC

The strip has 3 separate indicator dye color bands. The combination of color change resulting from each test is compared with the color-coded comparison chart, printed on the dispenser, giving improved speed and accuracy in reading.

#### Dispensers Type SR

A full range and some narrow ranges in this popular pH indicator dispenser.

#### Indicator Books

The book format is particularly suitable for educational and industrial use. In schools they are economical because the amount of paper per student can be carefully controlled.

## Acid-Alkali Test Papers

#### Litmus Blue and Litmus Red

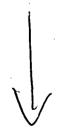
These easy-to-use test papers facilitate a general test for acid or alkaline reaction. The change occurs around pH 5 to 8. They are particularly recommended for educational use.

#### Congo Red

This test paper changes color from blue to red in the range pH 3 to 5 for the determination of neutralization point in strong acid/weak alkali reactions.

#### Phenolothalein

This white paper changes to pink at pH 8.3 and becomes red at pH 10. It is useful for the



determination of the neutralization point in weak acid/strong alkali reactions.

## **Specialized Test Papers**

#### **Lead Acetate Test Paper**

Used for detecting hydrogen sulfide, this rapid qualitative test paper when wetted with distilled water can detect as little as 5 ppm of  $\rm H_2S$  in the atmosphere or in a gas stream. Hydrogen peroxide can be detected with this paper by preblackening the paper in  $\rm H_2S$ . Concentrations as low as 4 ppm can be detected.

### Potassium Iodide Test Paper

Used for detecting chlorine and other oxidizing agents. In acid solution, oxidizing agents react with the iodide in the test paper to liberate iodine. The paper will turn blue in the presence of an oxidizing agent (e.g., Cl<sub>2</sub>, Br<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, HNO<sub>2</sub> etc.).

### **Universal Indicator Papers**

Universal indicator papers have been impregnated with a mixture of several indicators. On contact with the sample solution they assume a particular color. A check against the color comparison table supplied allows the pH to be determined.

Ordering Inform	nation - pH Indicators and Test Papers
Catalog Number	Description
Strips	
2613-991	Colour Bonded, 0.0 to 14.0 range, 6 x 80 mm, 100 strips, 1/pk
2614-991	Colour Bonded, 4.5 to 10.0 range, 6 x 80 mm, 100 strips, 1/pk
2612-990	Integral Comparison Strip, 1.0 to 12.0 range, 11 x 100 mm, 200 strips, 1/pk
2626-990	Integral Comparison Strip, 1.8 to 3.8 range, 11 x 100 mm, 200 strips, 1/pk
2627-990	Integral Comparison Strip, 3.8 to 5.5 range, 11 x 100 mm, 200 strips, 1/pk
2628-990	Integral Comparison Strip, 5.2 to 6.8 range, 11 x 100 mm, 200 strips, 1/pk
2629-990	Integral Comparison Strip, 6.0 to 8.1 range, 11 x 100 mm, 200 strips, 1/pk
2630-990	Integral Comparison Strip, 8.0 to 9.7 range, 11 x 100 mm, 200 strips, 1/pk
2631-990	Integral Comparison Strip, 9.5 to 12.0 range, 11 x 100 mm, 200 strips, 1/pk
Dispensers	
2611-628	Three Colours, Reel, 1.0 to 11.0 range, 10 mm x 5 m, 1/pk
2600-100A	Standard Full Range, Reel, 1.0 to 14.0 range, 7 mm x 5 m, 1/pk
2600-101A	Standard Narrow Range, Reel, 0.5 to 5.5 range, 7 mm x 5 m, 1/pk
2600-102A	Standard Narrow Range, Reel, 4.0 to 7.0 range, 7 mm x 5 m, 1/pk
2600-103A	Standard Narrow Range, Reel, 6.4 to 8.0 range, 7 mm x 5 m, 1/pk
2600-104A	Standard Narrow Range, Reel, 8.0 to 10.0.range, 7 mm x 5 m, 1/pk
Acid-Alkali Test Dispensers	Papers
2600-201A	Reel, Litmus Blue, 7 mm x 5 m, 1/pk
2600-202A	Reel, Litmus Red, 7 mm x 5 m, 1/pk
2600-204A	Reel, Phenophthalein, 7 mm x 5 m, 1/pk
Books	
2600-601	Book, Litmus Blue, 10 books of 20 strips, 10/pk
2600-602	Book, Litmus Red, 10 books of 20 strips, 10/pk
2600-500	Book, 1.0 to 11.0 range, 10 books of 10 strips, 20/pk

1-800-452-1261

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Catalog No. or Keyword

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Home > Apparatus & Technology > Laboratory Supplies > Test Papers > Assorted > Potassium Iodide Starch Test Papers, Strips in Vial



## Potassium Iodide Starch Test Papers, Strips in Vials

Price & Description

Catalog Number

Price Per Vial Price 12 Vials

AP7890 \$1.75 \$19.92

Product Description

Supplied in stoppered vials containing 100 strips per vial.

Used to test for peroxides and other oxidizing agents. Color varies from white through blue to black.

Test Papers, Strips in Vials AP7901, etc.

#### **Additional Resources**

No Additional Resources at the time

Flinn Catalog/Reference Manual Page 277

YOU MAY ALSO LIKE (10)

### **Similar Products**

Catalog No	Description	Use .	Per Vial	12 Vials .	· Qty
AP7901	Cobalt Chloride Test Papers, Strips in Vials	Paper indicates relative humidity by color change: pink indicates high humidity; blue indicates low humidity.	\$1.75	\$18.96	1 Add
AP7912	Congo Red Test Papers. Strips in Vials	For strong acids. pH 3.0 (blue-violet) to 5.0 (red)	\$1.45	\$16.56	1 Add
AP7893	Lead Acetate Test Papers, Strips in Vials	Used to test for hydrogen sulfide (H2S) vapors. Paper must be moistened with water prior to use.	\$1.30	\$14.88	1 Add
AP7923	Litmus Blue Test Papers, Strips in Vials	Used to test for acids. pH 4.3 (red) to 6.8 (blue-violet)	\$1.05	\$11.88	1 Add
AP7934	Litmus Neutral Test Papers. Strips in Vials	Used to test for acid or alkali.	\$1.05	\$11.76	1 Add
AP7945	Litmus Red Test Papers, Strips in Vials	Used to test for alkalı. 6.8 (blue-violet) to 8.1 (blue)	\$1.05	\$11.76	1 Add
AP7956	Methyl Orange Test Papers, Strips in Vials	pH 3.1 (red) to 4.4 (orange)	   <b>\$2</b> .15	\$24.48	1 Add

	<u>AP7967</u>	Methyl Violet Test Papers_Strips in Vials	pH 0.2 (yellow) to 2.0 (blue-violet)	\$1.75	\$19.92	1	Add
	AP7978	Phenolphthalein Test Papers, Strips in Vials	Very sensitive to ammonia vapors, pH 8.2 (colorless) to 10.0 (pink)	\$1.65	\$18.72	1	Add
:	AP7989	PTC (phenylthiocarbamide taste test paper) Test Papers, Strips in Vials	Used to test for the genetic ability to experience a bitter taste from PTC. Occurs in 70% of the population.	\$2.00	\$22.80 !	1	Add
	AP7891	Sodium Benzoate Taste Test Papers, Strips in Vials	Used to test for the genetic ability to experience a bitter taste from thiourea. Some individuals can taste either PTC or thiourea, but not both.		\$19.56 <u>`</u>	1	Add
	AP7892	Thiourea Taste Test Papers, Strips in Vials	Thiourea Taste Test. Used to test for genetic ability to experience a bilter taste from thiourea.	\$1.75	\$19.92	1	Add
	AP7990	Control Taste Test Papers, Strips in Vials	Used as an untreated control for taste tests with PTC, sodium benzoate, and thiourea.	\$1.05	<b>\$</b> 11.76	1	Add

## You May Also Like















## Questions in the Lab?



800-452-1261 Technical Support Available M-F 7:30a-5:00p CT

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Equipment	Manufacturer	Model Number	Comments
Freeze Packs	Coleman Trans Temp	40 411	from Ames Discontinued
Glassware	Pyrex, Kimax, Corning, Wheaton, Fisherbrand	Various Beakers, Flasks and Graduated Cylinders*	Borosilicate Glass *Several 100 mL Graduated Cylinders are Class "A"
Glassware Washer	Betterbuilt Forma Scientific	Turbomatic Jr. 8890	Not In Use
Hot Air Oven	Napco	430	
Immersion Oil	Weber (Cargille)	2030-01	Type B (Pint)
Incubators:  Walk-In  Environment	American Blower Corporation with GE Motor Lab-Line	M-77721-A 5HK33GGH 702	Water, SMA, VRB Water Over-flow
Laminar Flow Hood	Troemner	702	water Over-now
Lactek: Idetek Reader Jet Washer Mixer Timer	IDEXX IDEXX Thermolyne Gray Lab	Roto Mix Type 50800	
Media Dispensers	SEPCO Filamatic	Brewer 60454 AB-5	
Membrane Filter: Filters Funnels	Gelman Millipore	4242 HAWG 047 S1	
Microscopes	Bausch & Laumb Nikon	Flat Field Labophot	Back-Up
Microscope Slides	Applied Research Institute	E5144	Milk Smear 5 Circle
Milk Shipping Cases	Polyfoam Packers Polyfoam Packers Trans Temp*	390 422 System 512*	for Raw Milk for Pasteurized Milk Pasteurized Milk (*Discontinued)
Needle, Bent Point	Weber	2019-30	for DMSCC
pH Meter	Orion	420 A	

Equipment	Manufacturer	Model Number	Comments
pH Meter Printer	Orion	900 A	
Pipets/Pipettors/ Dispensers	Advanced Instruments Advanced Instruments Advanced Instruments Biohit Proline Falcon Falcon Falcon Fisherbrand Kimax Kimax MLA D-Tipper MLA D-Tipper MLA D-Tipper Pyrex Pyrex Stat Matic (IDEXX) Stat Matic II (IDEXX) VWR	FLA885 FLA825  710-500 7530 7502 7555 7529 13-671-102 M 37079 37079 1055C 1060C 7056 7086  Step-Pette (411)	75 μL (Fluorophos) 25 μL (Fluorophos) 2000 μL (Fluorophos) 100-5000 μL 10 mL (Disposable) 5.0 mL (Disposable) 2.2 mL (Disposable) 1.1 mL (Disposable) 1.1 mL (Disposable) 10 mL (in 1/10 mL) 11 mL 1.1 mL 100 μL 250 μL 1000 μL 11 mL (Red) 10 mL ( in 1/10 mL) Lactek 250 μL & 500μL Lactek 250 μL Repeater Pipettor
Pipet Accessories	MLA Nozzle Inserts Brinkman Hydrophobic Filter	8066 50-09-050-7	
Pipet Aides	Brinkman Pipet Helper Scienceware (Bel-Art)	50-09-000-1 F37886-0000	
Pipet Tips	Advanced Instruments Advanced Instruments Biohit Proline (Vangard) MLA MLA VWR	FLA880 374 (Tri-Continent Sci.) 780200 9025 9026 53499-809	Re-usable for 75 μL Re-usable for 25 μL up to 200 μL up to 1000 μL for Repeater Pipettor
Quanti Trays	IDEXX	WQT-2K (98-21675-00)	97 Well
Quanti Tray Sealer	IDEXX	2020 (WQTS-110)	:
Racks and Baskets	Fisher Fisher	14-966D 14-799-4 14-793-11	10x6x6 Aluminum Basket 10x6x6 Stainless Steel Basket 48 Place Wire Tube Rack 108 Place Wire Tube
	Fisher Nalgene	14-793-13 14-809-3	Rack 72 Place Blue Plastic Tube Rack 24 Place Wire Tube Rack

Equipment	Manufacturer	Model Number	Comments
	Scienceware (Bel Art)	F18788-1601	
Refrigerators: "7-11 Style" Walk-In	True BOHN	GDM-40 ADT0900F	
Sample Containers	Nalgene IDEXX	2105-0004 98-06159-00	For Colilert Test
SNAP: Image Reader	IDEXX		
Staining Dish	Weber Scientific	2006-00 2006-03 2006-06 2006-09	Complete Set Replacement Dish Replacement Cover Replacement Frame
Stereo Microscope	American Optical		
Stereo Microscope Light	Bausch & Laumb		
Stir/Hot Plates	Fisher Thermolyne	50800	
Stopwatch	VWR	62379-460	
i Syringe	Applied Research Institute	ARI Model A (ARIIII)	0.01 mL (DMSCC)
Thermal Paper: Fluorometer Idetek Reader	Advanced Instruments IDEXX	FLA835	5 Pack
Thermometers  Brooklyn  Brooklyn  Brooklyn  Ertco  Ertco (Advanced Instruments)  Fisher  Fisher  Fisher  Fisher  Fisher		1572 MX 6405 MX 3968C8 527 (20 - 45°C in 0.5°C) 15-041A 15-041C 15-043A 15-000B	Certified MRT In Use MRT's Certified (Hot Air Oven) Hot Air Oven Block Heater Certified (To 51°C) Certified (To 100°C) General Use (To 101°C)
Ultraviolet Light	Black Ray Spectroline	UVL-56 EA-160	Back-up
Water Baths  Blue M  NAPCO  Precision Scientific		Magni Whirl 230 A Thelco 184	Fecal Bath SMA/VRB Foss

Equipment	Manufacturer	Model Number	Comments
ĺ	Precision Scientific	. 182	Delvo
Water Bath Circulators	VWR	1112	
Water Purification System	Barnstead/Thermolyne Millipore Millipore	A1213 Super Q Milli-RO40	Still
Weights	Denver Fisher VWR (Troemner) VWR (Troemner)	2-219 80593050 80093830	for Analytical Balance Class S Class S (200.0 g) Class S (150.0 g)

Media/Reagents	Brand/Manufacturer	Part/Catalog Number	Amount
Amonium Hydroxide	EM Science	AX1303-13	500 mL
Bath Clear	Fisher	13-641-334	6 X 125 mL
Bromcresol Purple	Sigma	B5880	25 g
Bromothymol Blue 0.04%	Fisher	S122-500	500 mL
Brilliant Green Bile Broth	Difco	0007-17	500 g
Bronopol (2-Bromo-2-Nitro-1,3-Pr opanediol)	Sigma	B-0257	25 g
Buf-Fax Tablets (Stabilized)	Applied Research Institute	C175B	Qnty. 50
Butyl Alcohol	EM Science	BX-1780-5	4 L
Charm 0.008 IU Positive Control	Charm Sciences	PEN G 008-5	5 Pack (100 mL each)
Charm Zero Control Standard	Charm Sciences	:	10 Bottles (to make 100 mL each)
Colilert	IDEXX	WP200	200 Tests/Box
Colilert-18	IDEXX	WP200-18	200 Tests/Box
Delvo Test P 5 Pack	Gist-brocades (through Nelson Jameson)	2333480	5 Trays, 96 Wells/Tray with Nutrient Tablet
DPD Total Chlorine			

Media/Reagents	Brand/Manufacturer	Part/Catalog Number	Amount
Reagent Powder Pillows	Hach	14076	50 Pillows
Dri-Contrad	Fisher	04-355-6	12.5 Kg
EC Medium_	Difco	0314-17-2	500 g
Ethidium Bromide "Dye"	Foss Electric	P/N 342147	10 Tablets
Ethyl Alcohol	Aaper Alcohol & Chemical		373.5 lbs
Fluorophos Calibrator Set (A, B and C)	Advanced Instruments	FLA250	30 mL each of A, B and C
Fluorophos Reagent 225 Set with Cuvettes	Advanced Instruments	FLA225 (FLA220 without cuvettes)	(2) 240 mL Substrate (2) 240 mL Buffer
Indo-Phax Tablets (Stabilized)	Applied Research Institute	C181	Qnty. 50
Lactek Beta-Lactam Kit	IDEXX		20 Tube Kits
Lactek Ceftiofur Kit	IDEXX ·		20 Tube Kits
Lactek Milk Controls	IDEXX		(3) "+" Controls (3) "-" Controls
Lauryl Tryptose Broth	Difco	0224150	500 g
L W Stain (Original Tetrachloroethane)	Weber Scientific	2001-05	1 Qt.
Magnesium Acetate	Fisher	M13-500	500 g
Magnesium Chloride	Fisher	M33-500	500 g
mEndo LES Agar	Difco /	0739-17	500 g
Methanol	Fisher	A412-4	4 L
mFC Agar	Difco	0677-17-3	500 g
Nutrient Agar	Difco	001-17-0	500 g
Nutrient Broth	Difco	0003-17-8	500 g
Phosphatase Standards (Scharer Rapid)	Applied Research Institute	C-130	One each of 1, 2 and 5 Scharer Units
Phos-Phax Tablets	Applied Research	C171	Qnty. 50

Media/Reagents	Brand/Manufacturer	Part/Catalog Number	Amount
(Stabilized)	Institute		,
Plate Count Agar (Standard Methods Agar)	Difco	24794	500 g
Potassium DiHydrogen Phosphate	Fisher	P-285	3 Kg
Potassium Hydrogen Phthalate	Sigma	P-3792	500 g
Potassium Hydroxide	Sigma	P-6310	250 g
Sodium Hydroxide	EM Science	SX0590-13	500 g
Sodium Thiosulfate	Fisher	\$445-500	500 g
Somatic Cell Standards	Dairy Quality Control Institute (DQCI)	Somatic Cell	I Set of (2) L, (2) LM, (2) MH and (2) H
Staphene	Steris	6389-72	453 g (Can)
Triton X-100	Foss Electric	P/N 028860	100 mL
Tryptic Soy Broth	Difco	0370-17 3	500 g
Violet Red Bile Agar	Difco	211695	500 g
Wilkerson's Dryer's Silica	Wilkerson (Foss Electric)	Z 32 00086 .	0.88 lbs (400 g)

## II. Quality Control - Equipment and Reagents

Equipment	Program	Parameter	Frequency	Limits
Autoclave	Milk/Water	Sterilization Temperature	Each Run	120.0±1.0°C (Media) 132.0° (Waste)
		Sterilization Time	Each Run	12-15 min (Media) 30-60 min (Dil. Water)
1		Total Time	Each Run	45 min (Waste) ≤45 min (EPA) ≤60 min (FDA)
		Exhaust Time Sterilization Check Timer Check Preventive Maintenance	Each Run Weekly Quarterly	>10 min No Growth = Set Time
<u>,</u>		Preventive Maintenance	Bi Monthly	Acceptable to Service Provider

Equipment	Program	Parameter	Frequency	Limits					
Balance (Analytical)	Milk/Water	Calibrate with: 60g, 50g, 20g, 10g, 5g, 2g, 1g, 500mg, 200mg, 100mg, 50mg, 20mg, 10mg, 5mg, 2mg, Zero	Monthly	$\geq 100 \text{mg} = \pm 0.25\%$ $< 100 \text{mg} = \pm 5.0\%$					
	į į	Annual Service		Acceptable to Service provider					
Balance (Electronic)	Milk/Water	Calibrate with: 1000g, 200g, 200+0.1g, 150g, 100g, 50g, 10g, 1g, 0.1g, Zero	Monthly	± 0.25%					
·		Annual Service	Annually	Acceptable to Service Provider					
Colony Counter	Milk/Water	Clean	Monthly	Dust Minimized					
Conductivity Meter	Milk/Water	Calibration with 10µmhos Standard	Monthly	= 10 μmhos					
Fluorometer	Milk	"+" Control "-" Control AD Mode AD Mode with Calib "C"	Each Set-Up Each Set-Up Each Set-Up Each Set-Up	500±150 mu/L <20 mu/L 308 - 314 (_314) 600±20					
Fossomatic 90	Milk	Air Supply Transfer Pressure Rinsing Pressure Operating Pressure Vacuum Pressure Electronic Cell Count Dispenser Filling Time Intake Filling Time Syringe Reservoir Zero Count Standards Coefficient of Variation	Each Set-Up Hourly Each Set-Up	60 - 90 psi 0.2 Bars 1.5 Bars 3.0 Bars -40 to -60 kPa 4000 ± 4 4 - 5 sec 3 - 4 sec Half-Filled and Clean <5 <5 <15% from DMSCC (L), <10% from DMSCC (LM, MH, H) ±5% of Prev. Est. Value (in triplicate) 5%					
General Laboratory	Water/Milk	Temperature Lighting Air Quality	Daily Annually Each Plating	16°- 27°C > 50 foot-candles < 15 CFU/15min					

Equipment	Program	Parameter	Frequency	Limits .			
Glassware	Water/Milk	Detergent Residue	Each Load	None Detected (Green) with 0.04% Bromthymol Blue			
Hand Tally	Milk/Water	Accurate	Annually	100 = 100			
Hot Air Oven	Milk/Water	Sterilization Temperature Sterilization Time Sterility Check	Each Run Each Run Monthly	>170°C ≥ 2 hours at temp. No Growth			
Incubators: Walk-In Water Environette Fecal Water Bath SMA/VRB Walk-Ins* Delvo 5 Pack	Water Water Water Milk Milk	Temperature Recorded  *Moisture Weight Loss	Twice Daily Twice Daily Twice Daily Twice Daily Start/Finish *Quarterly	35.0±0.5°C 35.0±0.5°C 44.5±0.2°C 32.0±1.0°C 64.0±2.0°C * <15%			
Lactek	Milk	Timer Check R.P.M. Jet Washer	Quarterly Quarterly Quarterly	3 min = 3 min > 220 r.p.m. 10 mL/sec			
Membrane Filter: Funnels Filters	Water	100 mL Volume Sterility ("Pre") Sterility ("Filter")	Annually Each Run Each Run	100 mL ± 2.5 mL No Growth No Growth			
pH Meter	Milk/Water	Calibrate with 4,7 and 10	Each Use	Slope: 95-102%			
Pipets (Mechanical) / Dispensers	Water/Milk	Calibration	Quarterly	± 2.5% (EPA) ±5% (FDA)			
Quanti Tray Sealer	Water	Leaks	Monthly	None Detected			
Refrigerators	Water/Milk	Temperature Recorded	Twice Daily	0.0 - 4.4°C			
Sample Containers: Nalgene	Water	Sterility (1 Container per autoclave rack)	Each Run	No Growth			
Sample Containers: Colilert Bottles	Water	Sterility Volume Autofluorescence	Each New Lot	No Growth 100 mL ± 2.5 mL None Detected			
Screw Caps with Liners	Milk/Water	Non-Toxic	Each new batch/lot	No Growth Inhibition by Delvo P 5 Pack			
SNAP Reader	Milk	Performance Check Set:	Each Set-Up				

Equipment	Program	Parameter	Frequency	Limits
		Device 1 Device 2		0.65. ± 0.15 2.00 ± 0.50
Thermometers	Milk/Water	Calibration at temperature of use	Annually	≤0.5°C difference from reference thermometer
Thermometer (Reference)	Milk/Water	Ice Point (FDA)	Annually	0.0°C
(Reference)		Replace (EPA)	Every 5 yrs.	
UV Lamp	Water	Clean	Monthly	Clean with Ethanol
Weights	Wate/Milk	Accuracy	Annually	Acceptable to Service Provider

Media/Reagents	Program	Parameter	Frequency	Limits
Brilliant Green Bile Broth	Water/Milk	pH Sterility Productivity	Each Batch Each Batch Each Batch	7.2 ± 0.2 No Growth (Blank) Growth, No Gas (NLF) Growth, Gas ( <i>E. coli</i> )
Calibrators A, B and C	Milk	Calibration Ratio	Each Use	Ratio: 146 - 154
Chromogenic/Fluoroge nic Substrate (Colilert)	Water	Autofluorescence Productivity	Each Lot Each Lot	None Detected No Yellow Color (Cloudy) - Pseudomonas; Yellow Color, No Fluorescence - Klebsiella; Yellow Color, Fluorescence - E. coli
Delvo P 5 Pack	Milk	"+/-" Controls	Each Day Samples are Run and with Each Confrimation	Appropriate Results
Dilution Water	Water Milk*	*pH Sterility *Sterility *Toxicity	*Each Batch Each Batch *Each Plating Quarterly	*7.2 ± 0.2 No Growth *No Growth *< 15% Reduction in count from 0 to 45 min
Dishwasher Detergent	Milk/Water	Inhibitory Residue Test	Each Lot or at	<15% difference in Avg. Counts between

Media/Reagents	Program	Parameter	Frequency	Limits		
			least Annually	Group s A and D		
EC Medium	Water	pH Sterility Productivity	Each Batch Each Batch Each Batch	6.9 ± 0.2 No Growth (Blank) Growth, No Gas (NLF); Growth, Gas (E. coli)		
Lactek BL and CEF	Milk	"+/-" Controls  Wash Water	Each Day Samples are Run and with Each Confrimation Each Use	Appropriate Results  Clean and Clear		
Lauryl Tryptose Broth (2X*, 1.5X and 1X)	Water	pH Sterility Productivity	Each Batch Each Batch Each Batch	6.8 ± 0.2 No Growth (Blank) Growth, No Gas, *No Acid (NLF) Growth, Gas, *Acid (E. coli)		
mEndo LES Agar	Water	pH Sterility ("Media") Productivity	Each Batch Each Plating Each Plating	7.2 ± 0.2 No Growth Metallic Green Sheen with "+" Control		
mFC Agar	Water	pH Sterility ("Media") Productivity	Each Batch Each Plating Each Plating	7.4 ± 0.2 No Growth Metallic Green Sheen with "+" Control		
Nutrient Broth	Milk	pH Sterility	Each Batch I Bottle, Each Use	6.8 ± 0.2 No Contamination		
Penase Concentrate	Milk	Productivity	Each Run Used	With "+" Control = Yellow With "-" Control = Yellow		
Plate Count Agar	Water/Milk	pH Sterility	Each Batch Each Flask Used	7.2 ± 0.2 No Contamination		
Reagent Grade Water (Microbiologically Suitable (MS) Water)	Water/Milk	Conductivity Pb, Cd, Cr, Cu, Ni, Zn  Total Chlorine	Monthly Annually Monthly	< 2µmhos/cm at 25°C Each ≤0.05 mg/L Total ≤0.1 mg/L <0.1 mg/L		

Media/Reagents	Program	Parameter	Frequency	Limits
		Heterotrophic Plat Count	Monthly	<500 CFU/mL
Rinse Water	Water	Sterility	Each Batch	No Growth
SNAP	Milk	"+/-" Controls	Each Day Samples are ; Run and with Each Confirmation	Appropriate Results
Violet Red Bile Agar	Milk	pH Sterility	Each Batch Each Flask Used	7.4 ± 0.2 No Contamination

## III. Suppliers and Distributers

Supplier/Distributer	Phone Number
Advanced Instruments	1-800-225-4034
Applied Research Institute (ARI)	1-888-324-7900
Dairy Quality Control Institute (DQCI)	1-612-785-0484
Fisher	1-800-766-7000
Foss Food Technology (Foss Electric)	1-612-941-8870
IDEXX	1-800-321-0207
Nelson Jameson	1-715-387-1151
Sigma	1-800-325-3010
STERIS Corporation (formerly AMSCO) Service/Maintenance (Doug Hunt)	1-800-548-4873 (304) 540-2771 (Pager)
VWR Scientific Products	1-800-932-5000
Weber Scientific	1-800-328-8378

## IV. Equipment Manuals

See Attachments

## Chromogenic/Fluorogenic Substrate Test (Quanti Tray)

## 1.0 Introduction -

Colilert Reagent is used for the simultaneous detection and conformation of total coliforms and  $E.\ coli$  in water, which is based on the Defined Substrate Technology (DST). DST utilizes indicator-nutrient which cause target microbes contained in the sample and incubated in the DST reagent system to produce a color change (or another signal i.e., fluorescence), both indicating and confirming their presence. Coliforms as defined by this method are all bacteria that possess the enzyme  $\beta$ -D-galactosidase with  $E.\ coli$  also possessing the enzyme  $\beta$ -glucuronidase. The enzyme in Coliform will cleave the Chromegenic Substrate of the Test Reagent releasing the Chromogen just as the enzyme in  $E.\ coli$  will cleave the Fluorogenic Substrate releasing a fluorogen.

By utilizing the Quanti Tray System and 97 well trays, an estimation of coliform and *E. coli* density ranging from < 1 to > 2,419.2 can be determined from a single 100 mL sample portion. Higher counts can be determined by diluting the sample and multiplying the result by the appropriate dilution factor.

## 2.0 Sample Requirements-

## 2.1 Acceptance Criteria

- 2.1.1 For Compliance Samples: Maximum allowable elapsed time between sample collection and sample analysis is thirty (30) hours.
- 2.1.2 For Non-Compliance: Maximum allowable elapsed time between sample collection and sample analysis is forty eight (48) hours.
- 2.1.3 For Raw Source Waters (Surface, Ground, Spring): Maximum allowable elapsed time between sample collection and sample analysis is (8) hours. Temperature of receipt must be <10°C. If sample exceeds the 8 hours and 10°C, it is to be analyzed; however, "NOT VALID FOR SDWA COMPLIANCE REPORTING" must be checked under laboratory remarks. Samples exceeding 30 hours are not to be analyzed.
- 2.1.4 LT2 Monitoring (E. coli): Maximum allowable elapsed time between sample collection and sample analysis is thirty (30) hours. Temperature of receipt must be less than 10°C

## 2.2 Rejection Criteria

- 2.2.1 Insufficient air space to facilitate mixing of sample.
- 2.2.2 Sample contains residual chlorine. (Blue flash appears)
- 2.2.3 Sample exceeds maximum allowable time requirements as stated above.
- 2.2.4 Information on the Water Bacteriological Report Form (EM-1) is insufficient. (No date or time of collection)
- 2.2.5 Sample container was not furnished by the Office of Laboratory Services.

## 3.0 Sample Types -

- 3.1 Source Waters (Surface, including LT2 Samples; Ground; Spring and Bottled)
- 3.2 Dairy Farms
- 3.3 Sewage Suspects
- 3.4 Recreational Waters (Bathing Beaches Summer 2001)
- 3.5 Any Sample Requiring a Total and E. coli count
- 3.6 Swimming Pools requiring an estimation of coliform density
- 3.7 Flood/Disaster Samples requiring an estimation of coliform density

## 4.0 Reagents and Equipment -

- 4.1 For Analysis:
  - 4.1.1  $35.0^{\circ} \pm 0.5^{\circ}$ C Incubator. (Walk-In or Environette)
  - 4.1.2 Long wavelength (366 nm) Ultraviolet Lamp.

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	4.1.3	Clear, sterile, non-fluorescent 120 mL bottle. (Graduated a the 100 mL mark)  Colilert (or Colilert-18) Reagent.  70% Ethanol  Quanti Trays (97 well)  Quanti Tray Sealer  90 mL Sterile Water Dilution Blanks (If 10 <sup>-1</sup> Dilutions Ar Required)  99 mL Sterile Water Dilution Blanks (If 10 <sup>-2</sup> Dilutions Ar Required)  1.10 mL Sterile Pipets (Samples Requiring a 10 <sup>-1</sup> Dilution)							
	4.1.4	Clear, sterile, non-fluorescent 120 mL bottle. (Graduated at the 100 mL mark)							
	4.1.5	Colilert (or Colilert-18) Reagent							
	4.1.6	70% Ethanol							
	4.1.7	Quanti Trays (97 well)							
	4.1.8	Quanti Tray Sealer							
	4.1.9	90 mL Sterile Water Dilution Blanks (If 10 <sup>-1</sup> Dilutions Are Required)							
	4.1.10	99 mL Sterile Water Dilution Blanks (If 10 <sup>-2</sup> Dilutions Are Required)							
	4.1.11	10 mL Sterile Pipets (Samples Requiring a 10 <sup>-1</sup> Dilution)							
	4.1.12	1.1 mL Sterile Pipets (Samples Requiring a 10 <sup>-2</sup> Dilution)							
4.2	For Qu	ality Control:							
	4.2.1.	Quanit Cult Organisms:							
		1.2.1.1 Pseudomonas aeruginosa							
		1.2.1.2 Klebsiella pneumoniae							
		4.2.1.3 E. coli							
	4.2.2	Tryptic Soy Broth. (TSB) (Single Strength and Double Strength)							

## 5.0 Procedure -

5.1 General Procedures (100 mL and Dilutions)

4.2.3 Brom Cresol Purple Solution

- 5.1.1 Turn on Quanti Tray Sealer. Green light will come on when sealer is ready (approximately 20 minutes).
- 5.1.2 Sanitize area with 70% Ethanol and wash hands.
- 5.1.3 Record sample temperature on EM-1 Report form with infrared thermometer (LT2 E. coli samples only).
- 5.1.4 Shake sample 25 times in 7 seconds with a 1 foot movement.
- 5.1.5 Determine the appropriate dilution from the following table:

tion S	nple Type
100 mL Volume   1   2   3   4   5   6   7	Raw Source Waters (Ground) Raw Source Waters (Springs) Raw Source Waters (Bottled Waters) Raw Source Waters (LT2 Monitoring) Dairy Farms Recreational Waters Drinking Water (Public or Private) Requiring a Count
1 2	Raw Source Waters (Surface) Raw Source Waters (LT2 Monitoring)
1	Sewage Suspects and Ditches where high counts are expected. If unsure, a full 100 mL and 10-2 may be run on the same sample giving a range of <1 to > 241,920.
	expected. If unsure, a full 100 mL and 10-2 run on the same sample giving a range of

- \*Each LT2 Monitoring Sample requires 3 separate dilutions.
  - 5.2 For 100 mL:
    - 5.2.1 Remove excess sample by pouring off or removing it with a 10 mL sterile pipet so that only 100 mL remains.
    - 5.2.2 Add 1 packet of Colilert Reagent and shake to dissolve completely.

- 5.2.3 Label back of 97 well tray with the Lab Number, Date, Time and Dilution Factor (1X for 100 mL portion).
- 5.2.4 Pour sample into 97 well tray and tap on small wells to dislodge air bubbles.
- 5.2.5 Place tray in the rubber mold on the Quanti Tray Sealer and press "Seal".
- 5.2.6 Place in 35.0°±0.5°C incubator for 24 to 28 hours (27 to 28 perfered).
- 5.3 For 10<sup>-1</sup> Dilutions:
  - 5.3.1 Follow steps 5.1.1 thru 5.1.5 from above.
  - 5.3.2 Add one packet of Colilert to a 90 mL sterile water blank, cap and shake to dissolve the reagent.
  - 5.3.3 Pipet 10.0 mL of sample into the 90 mL sterile water blank.
  - 5.3.4 Shake 25 time, in 7 seconds with a one foot movement.
  - 5.3.5 Label back of 97 well tray with the Lab Number, Date, Time and Dilution Factor (10X for 10<sup>-1</sup> dilution).
  - 5.3.6 Pour sample into 97 well tray and tap on small wells to dislodge air bubbles.
  - 5.3.7 Place tray in the rubber mold on the Quanti Tray Sealer and press "Seal".
  - 5.3.8 Place in 35.0°±0.5°C incubator for 24 to 28 hours (27 to 28 perfered).
- 5.4 For 10<sup>-2</sup> Dilutions:
  - 5.4.1 Follow steps 5.1.1 thru 5.1.5 from above.
  - 5.4.2 Add one packet of Colilert to a 99 mL sterile water blank, cap and shake to dissolve the reagent.

- 5.4.3 Pipet 1.0 mL of sample into the 99.0 mL sterile water blank.
- 5.4.4 Shake 25 time, in 7 seconds with a one foot movement.
- 5.4.5 Label back of 97 well tray with the Lab Number, Date, Time and Dilution Factor (100X for 10<sup>-2</sup> dilution).
- 5.4.6 Pour sample into 97 well tray and tap on small wells to dislodge air bubbles.
- 5.4.7 Place tray in the rubber mold on the Quanti Tray Sealer and press "Seal".
- 5.4.8 Place in 35.0°±0.5°C incubator for 24 to 28 hours (27 to 28 perfered).
- 5.5 Reading, Interpreting and Reporting
  - 5.5.1 Remove the trays from the incubator after 24 hours incubation. Samples must be removed from the incubator with 28 hours. Because the sample is divided into 97 portions, some of the wells are slower to develop the color change. Therefore, it is preferable to let the trays incubate 27-28 hours.
  - 5.5.2 Examine each well on the tray for the presence of a yellow color (confirming the presence of coliform bacteria) that is equal to or greater than the compartor. Wells that are slightly yellow, but not as yellow as the comparator, must be place back into the incubator to incubate for the full 28 hours. Samples left in the incubator for more than 28 hours must be reported as "Laboratory Accident" unless they are clear.
  - 5.5.3 Count the number of large wells (including the very large well at the top of the tray) and the number of small wells that have a yellow color equal to or greater than the comparator and record in the "Conf24" column on the "Colilert Bench Sheet" (Attachment #1) as follows: # Large Wells/# Small Wells \* Dilution Factor.
  - 5.5.4 All trays that contain at least one yellow well (Total Coliform Positive Samples) must be taken into the Walk-In Incubator and

checked for fluorescence with the 366 nm UV light. Wells with fluoresce equal to or greater than the comparator are Positive for *E. coli* and must be marked with a marker.

- 5.5.5 Count the number of large wells and the number of small wells that fluoresce equal to or greater than the reference comparator and record in the "E. coli" column on the "Colilert Bench Sheet" (Attachment #1) as follows: # Large Wells / # Small Wells \* Dilution Factor.
- 5.5.6 Using the IDEXX Quanti-Tray/2000 MPN Table (Attachment #2) determine the number of total coliforms and *E. coli* as follows:
  - For total coliforms read down the chart for the number of large yellow wells and across the top for the number of small yellow wells. The point where the column and row intersect is the MPN value for total coliforms based on 100 mL of sample. If a 10<sup>-1</sup> dilution of the original sample was made the MPN value must be multiplied by 10. If a 10<sup>-2</sup> dilution of the original sample was made the MPN value must be multiplied by 100. Record the computed value as it appears on the chart in the "Total Coliform" column of the Colilert Bench Sheet.
  - For *E. coli* read down the chart for the number of large fluorescing wells and across the top for the number of small fluorescing wells. The point where the column and row intersect is the MPN value for total coliforms based on 100 mL of sample. If a 10<sup>-1</sup> dilution of the original sample was made the MPN value must be multiplied by 10. If a 10<sup>-2</sup> dilution of the original sample was made the MPN value must be multiplied by 100. Record the computed value as it appears on the chart in the second "E. Coli" column of the Colilert Bench Sheet.
  - 5.5.6.3 If all wells are clear (Negative for Total Coliforms) then record as "0/0 \* Dilution Factor" in the "CONF/COLI" column on the "Colilert Bench Sheet" and report as < minimum detection limit in the

"TOTAL" column. Minimum Detection Limits are as follows: < 1 for 100 mL portions, < 10 for 10<sup>-1</sup> dilutions and < 100 for 10<sup>-2</sup> dilutions.

- 5.5.7 Special Instructions for Computing and Reporting E. coli for LT2 Samples:
  - Since three different volumes (100 mL, 10, mL and 1.0 mL) of the same sample, the volume that yields the number of positive wells (# of Large Wells + # of Small Wells) in the countable range of 39 to 78 (40 to 80%) is the one that is to be used for reporting.
- 5.5.8 Record the date reported in the "Rpt Date" column and the initials of the analysts reading the results in the "Initials." column. Also, record the time the samples are read in the space to the right of the last column labeled "Read Time".

Note: The report date, analysts initials and time read may be recorded on the top line and then arrows drawn down. See example on Attachment #1.

- 5.5.9 After the data has been entered on the bench sheet for a particular sample then the Water Bacteriological Report Form (EM-1) is to be completed. Total Coliforms are to be marked as "Present" or "Absent". *E. coli* only has to be marked as "Present" or "Absent" if Total Coliforms are Present. The MPN value for total coliforms and *E. coli* is to then be recorded in the "\_\_\_\_\_\_ per 100 mL" space.
- 5.5.10 After all EM-1 forms are marked, they are to be placed in the basket labeled "Forms To Be Checked".
- 5.5.11 All forms are to be checked by a Microbiologist II or higher. All samples with Total Coliform Positive Results must be initialed by the analyst checking them on the bench sheet. Initials are to be placed to the right of the initials of the analyst reading the test. (See Attachment #1).

## 6.0 Quality Control

- 6.1 Colilert Quality Control
  - 6.1.1 See procedure Chromogenic/Fluorogenic Substrate Test (Colilert 100 mL), Section VI.
- 6.2 Additional Quality Control for the Quanti Tray:
  - 6.2.1 On a monthly basis, add 100 mL of a bromcresol purlple solution to a 97 well tray and seal. Check for any leaks and record the results on the "Quanti Tray Sealer Leak Check" form (Attachment #3)

Environmental Microbiology SOP / QA Manual Procedure: Colilert Quanti Tray Rev. 10/2006

## Attachment #1 Colilert Bench Sheet

## BENCH SHEET- COLILERT

0467	2.4	MACON	10/16/2000	10/17/2000		Transer	301 6 30 8 8				E. Coli				
6467	3A	MASON	10/16/2006			HITE				i		10/18	Dg.	17,	20. Pin
	3A	ROANE		10/17/2006		PARSONS					J				\ 
6469	3A	MASON	10/16/2006	10/17/2006		HITE		) 1,	!			:		<u>l</u> ,	e/
6470	81	PUTNAM	10/16/2006	10/17/2006		LYONS 101		49/29	18/0	5794	218			i	0
6471	3A	PUTNAM	10/16/2006	10/17/2006		LYONS									
6472	3A	PUTNAM	10/16/2006	10/17/2006		LYONS		Í	1.		<u> </u>				
6473	81	PUTNAM	10/16/2006	10/17/2006		TAONS 101		49(30	UZ	6131	305				- un
6474	3A	PUTNAM	:10/16/2006	10/17/2006		LYONS	4.50	١	1		;			1	
6475	3A	PUTNAM	10/16/2006	10/17/2006	The second	LYONS	111								
6476	3A	PUTNAM	10/16/2006	10/17/2006		LYONS			i :					1	
6477	3 <b>A</b>	PUTNAM	10/16/2006	10/17/2006		LYONS			:						
6478	3A	FAYETTE	10/16/2006	10/17/2006		BLAKE	1	:						- 1	
6479	3A	JACKSON	10/16/2006	10/17/2006		κv	1								
6480	3 <b>A</b>	MASON	10/16/2006	10/17/2006		HITE	100	;	i i						*****
6481	3 <b>A</b>	WYOMING	10/16/2006	10/17/2006		ENGLAND					1				-
	3A	WYOMING	10/16/2006	10/17/2006		SB								;	1
	3A	FAYETTE	10/16/2006	10/17/2006		BLAKE	54								
6484	3A	ROANE	10/16/2006	10/17/2006		PARSONS						.			
5485	38	MASON	10/16/2006	10/17/2006		WAUGH		+	11	q	Α			ļ.	<b>ار</b> ه
3486	3A	MASON	10/16/2006	10/17/2006		HITE	1.	<i>†</i>		Ď	A				Cin
6487 -	3A	MASON	10/16/2006	10/17/2006		HITE	17.3		-					}	-
6488 ·	3A	ROANE	10/16/2006	10/17/2006		STARCHER					:			. <del></del>	
5489	3A	MERCER	10/16/2006	10/17/2006		BELCHER							+		
	3 <b>A</b>	MERCER	10/16/2006	10/17/2006		BELCHER					:	\	1		1
3491	3A	MERCER	10/16/2006	10/17/2006		BELCHER	1	!		ATA: 00.000 AT	•				-
6492	3A .	LOGAN	10/16/2006	10/17/2006		MOUNTS	2,725							<u> </u>	
6493	3A	LOGAN	:10/16/2006			MOUNTS						···· /			1

Note: "Name/Co and Sample Lo" have been hidden to protect privacy.

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## Attachment #2

	# Large								IDE	XX (	Quan		•	/200			able	(per 1	100ml)				-			
	Wells											#	Small	Wells	Positi	ve										
	Positive	0	1	2	3	4	5	6	. 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22 .	23	24
_	0	<1	1.0	2.0	3.0	40	5.0	6.0	7.0	8,0	9.0	10.0	11.0	12.0	13.0	14.1	15 1	18.1	17.1	1.5.1	19.1	20.2	21.2	22.2	23.3	24.3
	2	10 20	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	18.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
	3	3.1	3.0 4.1	4.1 5.1	5.1 6.1	6.1 7.2	7.1 8.2	8.1 9.2	9.2 19.3	10.2 11.3	11.2 12.4	12.2 13.4	13,3 14,5	14.3 15.5	15,4 18,5	16.4 17.6	17.4 18. <del>0</del>	18.5 19.7	19.5 20.8	20.6 21.8	21.6 22.9	22.7 23.9	23.7 25.0	24.5 26.1	25.5 27.1	26.9 26.2
	ă	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11,4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
	5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23,3	24.4	25.5	28,6	27.7	28.5	29.9	31.0
-	6	6.3	7.4	a.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	15.1	19.2	20.3	21.4	22.5	23.6	24.7	25.3	26.9	29.0	29.1	30.2	31.3	32.4
	7	7.5	9.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	16.3	19.4	2D.5	21.8	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31,6	32.5	33.9
ı	8	5.6	9.7	10,8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.0	24.1	25.2	26.3	27.4	8.52	29.7	30.8	32.0	33.1	34.3	35.4
1 :	9 10	9.8 11.0	10.9 12.1	12.0 13.2	13.1 14.4	14.2 15.5	15.3 16.6	16.4 17.7	17.6 12.9	15.7 20.0	19.8 21.1	20.9 22.3	22.0 23.4	23.2	24.3	25.4	28.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.5	37.0
1 -	11	112.2	13.4	14.5	15.6	18.8	17.2	19.1	20.2	21,4	22.5	23.7	24.8	24.8 26.0	25.7 27.2	26.9 28.3	28.0 29.5	29.2 30.7	30.3	31.5 33.0	32.7	33.8 35.4	35.0 35.6	35.2 37.5	37.4 39.0	38.6 40.2
	12	13.5	14.6	15.8	16.2	18.1	19.3	20.4	21.8	22.5	23.9	25.1	. 28.3	27.5	28.6	29.8	31.0	32.2	33.4	34,6	35.8	37.0	35.2	39.5	40.7	41.B
Π	13	14.8	18.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	28.6	27.8	29.0	30.2	31.4	32.6	33.8	35,0	36,2	37.5	38.7	35.8	41.2	42.4	43.8
1 !	14	18.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	28.9	25.1	29.3	3D.5	31.7	33.0	34.2	35.4	36.7	37.₽	39.1	40.4	41.6	42.9	44.2	45.4
1	15	17.5	18 7	19.9	21.1	22.3	23.5	24.7	25.0	27,2	26.4	29.6	30.0	32 1	33.3	34.6	35.8	37.1	38.4	39.8	40,£	42.2	43.4	44.7	46.0	47.3
	16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	29,7	30.0	31.2	32.5	33.7	35.0	38.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
	17	20.3	21 B 23.1	22.8	24.1 25.6	25.3	20.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	38.7	38.0	39.3	40.6	41.9	43.2	44.5	45,9	47.2	48.5	49.6	51.2
	18 19	21.8 23.3	24.6	24.3 25.9	27.2	26,9 28,5	28.1 29.8	29.4 31.1	30.7 32.4	32.0 33.7	33.3 35.0	34.6 35.3	35.9 37.6	37.2 39.0	38.5 40.3	39.8 41.6	41.1 43.0	42.4 44.3	43.8 45.7	45.1 47.1	46.5 48.4	47.3 49.8	49.2 51.2	53.5 52.6	51.9 54.0	53.2 55.4
	20	24.9	26.2	27.5	28.8	30, 1	31,5	32.5	34.1	35.4	38.6	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
-	21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.0	37.3	35.6	40.0	41.4	42.6	44.1	45.5	46.9	48.4	49,8	51.2	52.6	54.1	55.5	55.9	58.4	59.9
	22	28.2	29.5	3D.9	32.3	33.6	35.0	38.4	37.7	39.1	49.5	41.0	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.5	59,3	60.5	62.3
	23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.0	45.4	48.8	48.3	49.7	51.2	52,7	54.2	55.6	57.1	52.6	60.2	81.7	€3.2	84.7
	24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55,0	56.5	58.0	59.5	ê1.i	62.6	64.2	85.9	67.3
-	25 26	33.6 35.5	35.0 36.9	35.4 35.4	37.9 39.9	39.3	49.8	42.2 44.3	43.7 45.9	45.2 47.4	48.9	48.2 50.4	49.7 52.0	51.2 53.5	52.7 55.1	54.3 58.7	55.8 58.2	57.3 59.8	58.9	60.5 63.0	62.0	63.6	85.2 87.9	66.6 62.6	68.4 71.2	70.0 72.0
	27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	58.0	57.8	59.2	60.8	62.4	64.1	65.7	67.4	69.1	79,5	72.5	74.2	75.9
	28	39.5	41.0	42.8	44.1	45,7	47.3	48.6	50.4	52.0	53.6	55.2	56.9	56.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
	29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	81.2	62.9	64.6	66.3	0.66	69,8	71,5	73.3	75.1	76.9	79.7	93.5	82.4
	30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	6D.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	60.2	\$2.1	64.0	85.9
	31	48.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	88.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	E.: 8	\$3.7	85.7	67.8	89.6
	32	48.7	50.4	52.1	53.8	56.6	57.3	59.1	60.9	62.7	84.5	66.3	66.2	70.0	71.9	73.8	75.7	77.8	79.5	81.5	83.5	85.4	67.5	62.5	91.5	93.6
	33 34	51.2 53.9	53.0 55.7	54.8 57.6	58.5 59.4	58.3 61.3	60.2 63.1	62.0 65.0	63.6 67.0	65.7 68.9	67,6 70.8	09.5 72.8	71.4 74.8	73.3 76.8	75.2 78.8	77.2 80.8	79.2 82.9	81.2 85.0	83.2 87.1	95.2 69.2	87.3 91.4	89.3 93.5	91.4	93.6 97.9	95.7 100.2	97.£ 102.4
	35	56.8	58.6	6D.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	8D.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	95.7 100.3	102.6	105.0	107,3
-	36	.50.8	61.7	63.7	65.7	67.7	69.7	71.7	73.5	75.9	75.D	8D.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	28.1	100.5	102.9	105.3	107.7	110.2	112.7
	37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.0	79.B	82.0	84.2	88.5	85.8	91.1	93.4	95.8	93.2	100.6	103.1	185.8	105.1	110.7	113.3	115.9	118.0
	38	68.3	68.4	70.6	72.7	74.9	77.1	79.4	81.8	63.9	88.2	88.8	91.0	93.4	95.8	98.3	100.8	103.4	105.₽	106.6	111.2	113 9	118.6	119.4	122.2	125.0
	39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	68.0	5E.4	90.9	93.4	95.9	95.4	101.0	103.6	106.3	109.0	111.5	114.6	117.4	12D.3	123.2	126.1	129.2	132.2
1 -	40	73.8	78.2	78.5	80.9	83.3	£5.7	69.2	93.6	83.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	116.2	121.2	124.3	127 4	130.5	133.7	137.0	140.3
1 :	41 42	78.0 82.6	80.5 85.2	53.0 87.0	85.5 80.5	83.0 83.2	90.6 93.0	93.3 9.89	95.9 101.7	99.7 104.6	101.4	104.3 110.6	107.1	110.0	113.0	118.0	110.1	122.2	125.4	128.7 137.2	132.0	135.4	138.8	142.3	145.9	149.5
il	43	87.6	90.4	93.2	96.0	29.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	116.9 124.6	120.1	123.4 131.7	126.7 135.4	130.1 139.1	133.8 143.0	147.0	140.8 151.0	144.5 155.2	148.3 159.4	152.2 163.3	156.1 168.2	160.2 172.8
i	44	93.1	96.1	89.1	102.2	105.4	105.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	155.5	163.1	187.9	172.7	177.7	182.9	188.2
1.	45	99.3	102.5	105.3	109.2	112.6	118.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.0	162.4	187.4	172.6	175.0	183.5	189.2	195.1	201.2	207.5
1 -	46	106.3	102.5	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	148.7	151.5	156.5	101.6	167.0	172.5	178.2	164.2	190.4	195.8	203.5	210.5	217.8	225.4	233.3
	47	114.3	115.3	122.4	128.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	1€0.4	172.3	178.5	185.9	191,5	198.9	206.4	214.2	222.4	231.D	240.0	249.5	259.5	270.0
	48	123.9	128.4	133.1	137.9	143.0	146.3	153.9	159.7	185.8	172.2	178.9	128.0	193.5	201.4	209.8	218.7	228.2	235.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
,	49	135.5	140.5	146.4	152.3	156.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	249.1	261.3	275.5	200,9	307.6	325.5	344.8	365.4	387.3	410.6	435.2
,	6-63235-01						ļ		1																	

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						!					<b>-</b>	6	(0.00											
# Large	l							IDE	XX	∡uan	ITI- I T	ay®	/2006	) IVIF	'N I	abie	(per	(00ml)						
Wells						1					#	Small	Wells	Positi	ve									
Positive	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	_41	42	43	44	45	46	47	48
. 0	25.3	26.4	27.4	25.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.9	37 9	38.9	40.0	45.0	42.1	43.1	44.2	45.3	46 3	47.4	49.5	49.5
1 2	26.6	27.7 29.0	28.7 30 0	29.8 31.1	30.8 32.2	31.9	32.9 34.3	34.0 35.4	35.0 38.5	36.1 37.5	37.2 38.5	38.2 39.7	39.3 40.9	40.4 41.9	41.4 43.0	42.5 44.0	43.6 45.1	44.7 48.2	45.7 47.3	46.8 49.4	47.9 49.6	49.0 59.6	50.1 51.7	61.2 62.8
3	29.3	30.4	31.4	22.5	33.6	24.7	35.6	38.5	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	48.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.9	33.9	35.0	30.1	37.2	38.3	39.4	40.5	41.5	42.8	43.9	45.0	46.1	47.2	45 3	49.5	59.6	51.7	52.0	54 0	55.1	58.3
5	32.1	33.2	34.3	35.4	38.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47 7	48.9	50.D	51.2	52.3	53.5	546	55.8	5.6.9	58.1
6	33.5	34.7	35.9	38.9	38.0	39.2	40.3	41.4	42.6	43.7	44 9	46.0	47.1 /	48.3	49.4	50.6	51.7	52.0	54.1	55 2	58.4	57.6	58.7	59.9
7	35.0	36.2	37.3	39.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.9	50.6	51.2	52.3	53.5	54.7	55.9	57.1	59.3	59.4	eo.e	e1.8
8 9	36.5 38.1	37.7 39.3	38.9 40.5	40.0 41.6	41.2 42.8	42.3 44.0	43.5 45.2	44.7 46.4	45.9 47.6	47.0 48.8	48.2 50.0	49.4 51.2	50.6 52.4	51.8 53.8	53.0 54.9	54.1 58.0	55.3 57.2	56.5 58.4	57.7 59.7	59.0 60.9	60.1 62.1	61.4 63.4	62.6 64.6	63 8 65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	48.9	48.1	49.3	50.6	. 51.9	53.0	54.2	55.5	56.7	57.9	59.2	80.4	61.7	82.9	64.2	65.4	68.7	87.9
11	43.4	42.6	43.9	45.0	48.3	47.5	49.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	81.2	82.4	83.7	85.0	68.3	67.5	68.6	70,1
12	43.1	44.3	45.6	46.8	49.1	493	50.6	51.8	53.1	54.3	55.8	56.9	58.1	59.4	80.7	62.0	63,2	64.5	85.8	57.1	€9.4	69.7	71.Q	72.4
13	44.9	46.1	47.4	49.0	49.0	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	81.5	52.9	64 1	65.4	66.7	69.0	89.3	79.7	72,0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53 1	54.4	55.7	57.0	58.3	59.8	60.9	62.3	63.6	64.9	88.3	67.6	86.9	70.3	71,6	73.0	74.4	75.7	77.1
15	46.6 50.5	49.9 51.8	51.2 53.2	52.5 54.5	53 8 55 8	55.1 57.2	58.5	57.8 59.9	59.1 61.2	60.4	61.9 64.0	63.1 65.3	84.5 86.7	65.8 68.1	67.2	68.5 70.9	69.9 72.3	71.3	72.6 75.1	74.0 78.5	75.4 77.9	78.8 79.3	78.2 80.8	79.6 82.2
17	52.5	53.8	55.2	58.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	89.1	70.5	71.9	73.3	74 8	76.2	77.6	79.1	77.9 80.5	62.0	83.5	64.9
18	54.8	56 D	57.4	59.8	60.2	616	63.ū	64.4	65.8	87.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	75.8	80.3	81.8	83.3	84.8	66.3	87.8
19	56.9	58.2	59.6	61.0	62.4	63.9	65.3	888	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	815	83.1	84.6	88.1	e7.6	89.2	90.7
20	59.0	60.4	81.9	63.3	64.8	66.3	67.7	89.2	70 7	72.2	73.7	75.2	76.7	76.2	79.8	81.3	82.8	84.4	859	87.5	69.1	99,7	92.2	93.6
21	513	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	78.4	77.9	79.5	91.1	92.6	84 2	85.8	87.4	89 0	90.6	92.2	8 89	95.4	97.1
22	53.9	65.3	66.9	68.3	69.8	71.4	72.9	74.5	78.1	77.6	79.2	80.8	52.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	86.3 68.9	67.9 70.5	69.4 72.1	71.0 73.7	72.5 75.3	74.1	75.7 78.6	77.3 60.3	78.9 51.9	8 <b>0</b> .5 83.6	62.2 66.2	93.9 86.9	55.4 58.6	87.1 90.3	98.7 92.0	90.4 93.8	92.1 95.5	93.8 97.2	95.5 99.0	97.2 100.7	99.9 102.5	100.6 104.3	102.4 106.1	104.1 107.9
25	75.7	73.3	75.0	75.6	78.3	80.0	81.7	63.3	85.1	6.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	108.3	105.2	110.0	111.9
26	74.5	76.3	76.0	79.7	E1.4	83.1	84.5	86.6	59.4	90.1	91.9	92.7	95.5	97.3	99.2	101.0	102.9	104.7	106 6	108.5	110.4	1123	114.2	118.2
27	77.6	79.4	81.1	82.9	24.6	88.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	195.9	106.9	108.8	1198	112.7	114.7	118.7	119.7	120.7
28	90.5	92.8	84.4	88.3	89.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	117.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	94.2	96.1	87.9	8.68	917	93.7	95.6	97.5	99.5	101 5	103.5	105.5	107.5	109.5	111.6	112.7	115.7	117.8	120.0	122 1	124,2	128.4	129.6	130.6
30	97.5	99.7	91,7 95,6	93.6 97.7	95.6 99.7	101.8	103.9	101.6 106.0	103.7	105.7	107.6	100.9	112.0	114.2	118.3	118.5	120.6	122.8	125.1	127.3	129,5	131.8	134.1	138.4
32	95.7	97.6	99.9	102.0	104.2	100.3	109.5	110.7	113.0	115.2	117.5	. 119.5	122.1	- 124.5	128.9	129.2	131.6	134.0	136.5	139.0	1415	144.0	146.6	149.1
33	100.0	102.2	104 4	108.6	109.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.6	130.3	132.9	135.3	137.9	140,4	143.0	145.8	149,3	150.9	153.7	158.4
34	104.7	107.0	109 3	111.7	114.0	116.4	119.0	. 121.3	123,5	126.3	128.5	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	159.8	161.5	164.4
35	109.7	112.2	114.6	\$17.1	i 19.6	122.2	124.7	127.3	129.9	132.8		138.0	140.5	143.5	148.4	149.2	152.1	- 155.0	158.0	181.0	164.0	187.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	129.4	131.1	133.9	138.7	139 5	142.4	145.3	148.3	151.3	154.3	157.3	180.5	163.6	166.8	170.0	1733	176.5	179 9	183.3
37	121.3	124.9 130.9	126.8 133.8	129.6 136.8	132.4	135.3 143.0	139,2 146,2	141.2 149.4	144.2 152.6	147 3	150.3 159.2	153.5	156.7	159.9	189 1 179.2	166.5 176.9	189.9	173.2	176.7	180.2	1937	187.3	191.0	194.7 207.7
38 39	127.9 135.3	138.5	141.7	145.0	132.9 148.3	143.0	155.1	158.6	162.1	155.9 165.7	159.2	162.6 173 1	166.1 178.9	169.6 160.7	194.7	198.7	190.4 192.7	194.2 196.8	188.0 201.0	191.8 205.3	195.7 209.6	199.7 214.0	203.7 219.5	207.7 223.0
40	143.7	147.1	150.6	154.2	157.8	181.5	165.3	169.1	173.0	177.0	181.3	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	2033	228.0	231,D	228.0	241.1
41	153 2	157.0	160.9	184 8	188.9	173.0	177.2	181.5	165,8	190.3	194.6	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245 8	251.5	257.2	263.1
42	154 3	168.6	172.9	177 3	181.9	186.5	191.3	198 1	201.1	206.2	211.4	216.7	222.2	227.7	232.4	239.2	245.2	251.3	257.5	263 8	270.3	278.9	283.6	299.5
43	177.5	182.3	187.3	192.4	197.6	202.9	209.4	214 0	219.8	225.6	231.6	238.1	244.5	251.0	257.7	264.6	271.7	27â.9	286.3	293.8	301,5	309.4	317.4	325.7
. 44	193.0	199.3	205 1	211.0	217.2	223.5	220.0	236.7	243.6	259.8	258.1	365.6	273.3	281.2	289.4	297.8	206.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	259.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.9	353.9	384.9	376.2	387.9	399.8	412.0	424.5	437.4
46 47	241.5 280.9	250.9 292.4	258.9 304.4	269.2 316.9	277.8 330.0	287.8 343.6	299.1 357.8	308.6 372.5	319.9 387.7	331.4 403.4	343.3 419.8	355 5 436 ð	368.1 454.1	381.1 472.1	394.5 490.7	408.3 509.9	422.5 529.8	437.1 550.4	452.0 571.7	467.4 593.8	483.3 618.7	499.6 640.5	519.3 865.3	533.5 691.0
48	250.a 344.1	350.9	378.4	395.8	416.0	436.0	458.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	699.3	721.5	755.6	791.5	829.7	870,4	913.9	960.6	1011.2
49	481.1	488.4	517.2	547.5	579.4	813.1	848.8	888.7	727.0	770.1	916.4	866.4	920.5	950.4	1046.2		1203.3		1413.6	1553.1	1732.9			>2419.6
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Environmental Microbiology SOP / QA Manual Procedure: Colilert Quanti Tray Rev. 10/2006

# Attachment #3 QUANTI TRAY SEALER LEAK CHECKS

Date	Sealer S/N (Model)	Observations	Q-Tray Lot#	Exp Date	Comments	Initials
	<b>1</b> 145135 (2020)	☐ Okay – No Leaks	Ì			
	□ 01557 (2X)	Do Not Use – Leaks Detected				
	145135 (2020)	Okay – No Leaks				
	□ 01557 (2X)	□ Do Not Use – Leaks Detected				
	145135 (2020)	Okay – No Leaks				
-	□ 01557 (2X) □ 145135 (2020)	☐ Do Not Use – Leaks Detected☐ Okay – No Leaks				+
	01557 (2X)	☐ Do Not Use – Leaks Detected	l	l		
	☐ 145135 (2020)	☐ Okay – No Leaks				
	□ 01557 (2X)	☐ Do Not Use - Leaks Detected				
	☐ 145135 (2020)	☐ Okay – No Leaks				
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				
	□ 145135 (2020)	□ Okay – No Leaks				
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				Ļ
	145135 (2020)	☐ Okay – No Leaks				
	01557 (2X)	☐ Do Not Use – Leaks Detected				<b>├</b> ──
	□ 145135 (2020) □ 01557 (2X)	<ul><li>Okay – No Leaks</li><li>Do Not Use – Leaks Detected</li></ul>	·			
	☐ 145135 (2020)	Okay – No Leaks				├
	01557 (2X)	☐ Do Not Use – Leaks Detected	-			
	145135 (2020)	☐ Okay – No Leaks	<del>                                     </del>			
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				ŀ
	☐ 145135 (2020)	☐ Okay – No Leaks	'		.	
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				
	☐ 145135 (2020)	☐ Okay – No Leaks				
	□ 01557 (2X)	Do Not Use – Leaks Detected				
	☐ 145135 (2020)	Okay - No Leaks				
·	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				
	145135 (2020)	☐ Okay – No Leaks				
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				<b>├</b>
	□ 145135 (2020) □ 01557 (2X)	☐ Okay – No Leaks ☐ Do Not Use – Leaks Detected				Ĭ
	145135 (2020)	☐ Okay - No Leaks			· .	<b>├</b> ──
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				1
	☐ 145135 (2020)	☐ Okay – No Leaks				<del>                                     </del>
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected			•	1
	☐ 145135 (2020)	☐ Okay – No Leaks				· ·
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				l
•	☐ 145135 (2020)	□ Okay – No Leaks				
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				
	145135 (2020)	☐ Okay – No Leaks				
	□ 01557 (2X)	☐ Do Not Use – Leaks Detected				<b>↓</b>
	145135 (2020)	Okay – No Leaks	4	, ,	,	
	□ 01557 (2X) □ 145135 (2020)	☐ Do Not Use – Leaks Detected☐ Okay – No Leaks				<del> </del>
	□ 01557 (2X)	Do Not Use – Leaks Detected	1			
	☐ 145135 (2020)	☐ Okay – No Leaks	,			<del>                                     </del>
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## **Heterotrophic Plate Count (HPC)**

#### I. Introduction -

The Heterotrophic Plate Count, formerly known as Standard Plate Count, is a method for enumerating heterotrophic bacteria in water samples. There are three (3) different methods for the Heterotrophic Plate Count: Pour Plate Method, Spread Plate Method and the Membrane Filter Method. The method that is used by the Environmental Microbiology Unit and the one described below is the Pour Plate Method. Bacterial colonies that are counted can arise from pairs, chains, clusters or single cells which are termed "Colony Forming Units (CFU's). This method can be used as an alternate method to Membrane Filtration and Multi Tube Fermentation when invalidation becomes a problem. Heterotrophic Plate Counts of >500 CFU's/mL are considered "Coliform Positive".

## II. Sample Requirements -

1. For Compliance Samples: Maximum allowable elapsed time between sample collection and sample analysis is eight (8) hours with a maximum transit time of six (6) hours and two (2) hours maximum processing time. If sample analysis cannot be analyzed within the eight (8) hours, then they may be refrigerated at < 4.0°C (Do Not Freeze) if examined within 24 hours.

For Non-Compliance: Begin analysis within 48 hours of collection.

- 2. Reject samples for any of the following reasons:
  - A. Insufficient air space to facilitate mixing of sample.
  - B. Sample contains residual chlorine.
  - C. Sample exceeds maximum allowable time requirements.
  - D. Information on the Water Bacteriological Report Form (EM-1) is insufficient. (No date or time of collection)
  - E. Sample container was not furnished by the Office of Laboratory Services

## III. Sample Types -

Samples analyzed by this method are done so by special request only. Such requests must include notifying the laboratory in advance of laboratory sample accession.

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Certain Bottled Water Companies request a Heterotrophic Plate Count in addition to their coliform test as a part of their Quality Control. These samples are considered Non-Compliance.

## IV. Reagents and Equipment -

## Reagents

- 1. Standard Methods Agar (also known as Plate Count Agar). Prepared by the Media Preparation Section and stored in 500 mL screw cap Erlenmeyer flasks (250 mL per flask) in the Media Preparation Refrigerator.
- 2.  $99.0 \pm 2.0$  mL Buffered Dilution Blanks. Prepared by the Media Preparation Section and stored in the Milk Room wall mounted cabinets. (Are used to accommodate dilutions higher than 0.1 mL)

## Equipment

- 1. Level Table with adequate Lighting (> 50 foot-candles)
- 2. Flowing Steam Chamber or Boiling Water Bath (to melt the media)
- 3. Circulating Water Bath  $(45.0 \pm 1.0^{\circ}\text{C})$
- 4. 1.1 mL Pipets
- 5. Pipet Bulbs
- 6. Petri Dishes (15 X 100 mm)
- 7. Incubator  $(35.0 \pm 0.5^{\circ}C)$
- 8. Partial Immersion Thermometer calibrated in the 44.0 to 46.0°C range
- 9. Darkfield Colony Counter
- 10. Hand Tally (for counting colonies)
- 11. Wax Pencil (for labeling plates)
- 12. 70% EtOH Cloths (cheese cloth dampened with 70% EtOH)

## V. Procedure -

## Set Up and Plating

Note: If HPCs are done on days that there are milk samples, then all of the control work is carried out by the Milk Room Personnel, only the HPC Plates will have to be labeled. If the samples requiring HPCs will also require a Coliform Test, then aseptically transfer 100 mL to the appropriate test vessel for the Coliform Test and

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- 1) Sample #, A, Dilution 1 (1.0 mL), Date
- 2) Sample #, A, Dilution 2 (0.1 mL), Date
- 3) Sample #, B, Dilution 1 (1.0 mL), Date
  - Sample #, B, Dilution 2 (0.1 mL), Date

## Plus the following Quality Control Plates:

- 1) Two (2) Air Quality Plates labeled "T-1" and "T-L"
- 2) One (1) plate labeled "H<sub>2</sub>O"
- 3) One (1) plate labeled "Pipet"
- 4) [Optional] One (1) plate labeled "SMA +"(On this plate, also include the "Name of the organism", "Volume Plated", "Date Plated" and the "Date the Agar Was Made"
- 6. Analysis may begin once the SMA Agar has reached the appropriate temperature (45.0 ± 1.0°C) and the temperature has been recorded on the work sheet. Pour 10 12 mL of SMA Agar into a plate labeled "T-1". Be sure that the agar covers the bottom of the plate. Place the "T-1" Plate in the middle of the plating area. Expose the plate to the air by removing lid and placing it beside the plate. Do not invert the lid (this would double the surface area exposed to the air). Set a timer for 15 minutes. Proceed with the next Step. When the timer goes off, place the lid on the plate, invert it and place it in the 35.0 ± 0.5°C incubator (the Environette). (Control plates must be kept with the sample plates unless, milk samples are also plated, in which case the control plates are kept with the milk samples)
- 7. Shake sample vigorously (25 times, within seven (7) seconds, with a one (1) foot movement)
- 8. With an alcohol cloth (cheese cloth with 70% EtOH), remove the sample bottle lid. Place the lid upside down in the cheese cloth on the table.
- 9. Remove the pipet from the pipet container (plastic bag or metal canister) without dragging the pipet tip across the exposed ends of the other pipets.
- 10. Insert the pipet no more than one (1) inch below the surface of the sample and draw the sample up above the 1.1 mL mark.
- 11. With the pipet at a 45° angle and the lower side of the pipet tip in contact with the neck of the sample container, adjust the sample volume in the pipet down to the

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take the remaining sample to the Milk Room for plating.

- 1. Turn on the Standard Methods Agar (SMA) water bath and circulator and set to  $45.0 \pm 1.0$ °C. (Bath should be allowed at least one (1) hour to reach temperature.)
- 2. Calculate the amount of SMA Media needed as follows:
  - A. Determine number of plates needed. There will be four plates per sample (1.0 mL and 0.1 mL in duplicate) plus two (1) plate for an air quality control (labeled "T-1") plus one (1) plate for the pipet sterility control plus one (1) plate for a water blank and one (1) plate for a positive control (0.5 mL from the "10-8" dilution of E. coli) [Positive Control is optional].
  - B. (Number of Plates X 15 mL agar per plate) ÷ 250 mL agar per flask = Number of Flasks Required [Round up to the next whole number and add two (2).
- 3. Obtain the required amount of SMA Media from the Media Preparation Section's Walk-In Refrigerator. Loosen the flask's lids and place them in the Flowing Steam Chamber. Turn on Steam Chamber and set a timer for 45 minutes.
- 4. When SMA Media is melted, remove from the Flowing Steam Chamber using heat resistant gloves, turn off Steam Chamber and place the media in the Tempering Bath (Step 1). Be sure the temperature of the Tempering Bath is  $45.0 \pm 1.0^{\circ}$ C, adjust if necessary. Replace the cap of the temperature control flask with foil and using a pen or pencil, make a whole large enough to insert a thermometer. Place a thermometer calibrated in the  $44.0 46.0^{\circ}$ C range into the temperature control flask. Be sure that when placing the media in the bath that the water level in the bath rises slightly above the surface of the media in the flask. (Tempering the media may take up to an hour.)
- 5. Prepare the plating table as follows:
  - A. Wipe the table with 70% EtOH.
  - B. Obtain a discard bucket from the corner cabinet below and just to the left of the SMA Tempering Bath.
  - C. Obtain 1.1 mL pipets and pipet bulb.
  - D. Label plates with wax pencil on the lids, not the bottoms each sample has four (4) plates (unless higher than 0.1 mL dilutions are to be performed):

#### 1.1 mL mark.

- 12. Lift the lid of the petri dish (start with the petri dish with the highest dilution volume in most cases 0.1 mL) up and slightly to the side. (Just enough to allow insertion of the pipet)
- 13. With the pipet at a 45° angle and the lower side of the pipet tip in contact with the bottom of the petri dish, deliver 0.1 mL of sample and replace the lid to the plate.
- 14. Lift the lid of the petri dish with the next lowest dilution (in most cases the 1.0 mL petri dish) just as in Step 12.
- 15. With the pipet at a 45° angle and the lower side of the pipet in contact with the bottom of the petri dish, deliver 1.0 mL of sample to the petri dish with a column drain of two (2) to four (4) seconds. Touch pipet off in a dry spot on the bottom of the petri dish (pipet at a 90° angle with respect to the bottom of the petri dish) and replace the lid.
- 16. Repeat Steps 10 thru 15 for the duplicate plates. (Plates labeled "B")
- Move the plates containing the sample aliquot to the table's edge. Remove SMA Flask from the tempering bath and wipe off excess water with a paper towel. Remove the cap to SMA Flask (when placing the cap on the table, invert it). Lift the lid of petri dish up and slightly to the side, pour 10 12 mL of SMA Agar, replace lid. Pour no more than four (4) petri dishes before mixing. Mix plates as follows: five (5) times clockwise, five (5) times up and back, five (5) times left and right and five (5) times counter-clockwise. Mixing velocity should be sufficient to ensure uniform sample distribution, but not high enough to cause splashing on the plate lid.
  - 18. Pour an SMA Agar control from each flask of agar that is used into the "SMA-1", "SMA-2" or "SMA-3" Plates, respectively using the residual aliquot from each flask remaining after pouring all possible plates from that flask.
  - 19. When all plates have been poured, obtain a sterile dilution blank and shake it vigorously as in Step 7. Pour 1.0 2.0 mL into "H2O" Plate.
- 20. Using a sterile pipet, deliver 1.1 mL of dilution water from the dilution blank to the "Pipet" Plate. Pour and mix the plate as described in Step 17 above.

- 21. [Optional] Obtain "Positive Control" from the Walk-In Refrigerator and plate 0.5 mL into the "SMA +" Plate. This control plate is prepared only after all other plate preparation has been accomplished in order to avoid contaminating the other plates.
- 22. Allow all plates to solidify, invert them, and stack them no more than four (4) high in a  $35.0 \pm 0.5$ °C Incubator (the Water Room Environette Incubator). All plates must be placed in the incubator within 10 minutes of pouring them.
- 23. Incubate all plates for  $48 \pm 3.0$  hours.

If Dilutions Higher Than 0.1 mL Are Required, Accommodate As Follows:

- 24. One (1) 99.0 mL Dilution Blank will be required for the 0.01 mL and 0.001 mL dilutions. A second dilution blank may be needed if higher than 0.001 mL dilutions are required, allowing for preparation of 0.0001 mL and 0.00001 mL dilutions.
- 25. Follow Steps 1 thru 9 above but with the following exception:
  - A. There will be at least two (2) to four (4) extra plates per sample, possibly more. (Plan on two (2) plates for each sample for each higher dilution)
  - B. Using a wax pencil, label the first dilution blank "Sample #" and "10<sup>-2</sup>". If a second dilution bland is required, label it "Sample #" and "10<sup>-4</sup>"
- 26. Insert pipet no more than one (1) inch below the sample surface and draw sample up past the 1.0 mL mark.
- 27. With the pipet at a 45° angle and the lower side of the pipet tip in contact with the neck of the sample container, adjust the volume down to the 1.0 mL mark.
- 28. Remove the stopper to the dilution blank (first dilution blank, 10<sup>-2</sup>) and with the pipet at a 45° angle and the lower side of the pipet tip in contact with the neck of the dilution blank, deposit 1.0 mL of sample into the dilution blank and replace the stopper. (If higher than 0.001 mL dilutions are required, then this dilution blank (10<sup>-2</sup>) is shaken and 1.0 mL is to be transferred to a second dilution blank (10<sup>-4</sup>) following the pipetting procedures as outlined in Steps 26 thru 28)
- 29. Each dilution blank is to be treated like a sample and plated as per Steps 7 thru 15

above. The original sample will accommodate the 1.0 mL and 0.1 mL dilutions, the first dilution blank will accommodate the 0.01 mL and 0.001 mL dilutions, the second dilution blank will accommodate the 0.0001 mL and 0.00001 mL dilutions, etc.

### Reading, Computing and Reporting Counts

- 1. After 48 ± 3 hours at 35.0 ± 0.5°C, examine all plates with the aid of a darkfield colony counter. Read and record all QC Data first ("SMA Sterility", "T-1", "Pipet", "+ Control" [if done], and "H2O" plates)
- 2. Count all colonies using a Hand Tally.
- 3. Record counts onto the bench sheet in their appropriate columns. There are columns for "1.0 mL", "0.1 mL" and "HPC Report". If higher dilutions were used, then Special Notations (the dilutions used) are entered above the counts. (See Attached Copy of Bench Sheets Attachment 1)
- 4. Compute counts as follows:
  - A. if only one dilution yields plates with 30 to 300 CFU's, compute the mean for that dilution as the basis for the HPC;
  - B. if both dilutions yield plates with 30 to 300 CFU's, average the mean count for each dilution as the basis for the HPC unless the count computed for the higher dilution is more than twice the count computed for the lower dilution. In the latter instance, use the lower computed count as the HPC;
  - C. if neither dilution yields plates with 30 to 300 CFU's, use the mean count for the dilution nearest to 300 as the basis for Estimating the HPC,
  - D. if both dilutions yield only plates with fewer than 30 CFU's, Use the mean count of the duplicates of the lower dilution to estimate the HPC;
  - E. if both dilutions yield plates with 0 CFU's, estimate the HPC and < 1 times the lowest dilution used;
  - F. if only one plate of one dilution contains 30 to 300 CFU's, when one plate of one dilution contains 30 to 300 CFU's and the duplicate contains more

than 300 CFU's, use both plates in computing the HPC;

- G. if one plate of each dilution contains 30 to 300 CFU's and the duplicate contains more than 300 or less then 30 CFU's, use all four in computing the HPC;
- H. if both plates of one dilution contain 30 to 300 CFU's and only one duplicate of the other dilution contains 30 to 300 CFU's, use all four plates in computing the HPC unless excluded by "Spreaders" or "Laboratory Accidents";
- I. if both plates of the highest dilution have more than 300 CFU's but fewer than 10 CFU/cm<sup>2</sup>, use the plates with the highest dilution. Count 12 cm<sup>2</sup> and divide by 12 to determine the average per cm<sup>2</sup>. Multiply the average by the Plate Factor to determine the CFU/plate. Plate Factor is the area of the plate in cm<sup>2</sup>. ( $\pi$ r<sup>2</sup>). Average the plates and multiply by the dilution factor;
- J. if both plates of the highest dilution have more than 300 CFU's but fewer than 100 CFU/cm<sup>2</sup> and more than 10 CFU/cm<sup>2</sup>, Use the plates with the highest dilution. Count 4 cm<sup>2</sup> and divide by 4 to determine the average per cm<sup>2</sup>. Multiply the average by the Plate Factor to determine the CFU/plate. Average the plates and multiply by the dilution factor;
- K. if both plates of the highest dilution have more than 300 CFU's and more than 100 CFU/cm<sup>2</sup>, use the plates with the highest dilution. Estimate the HPC as > 100 times the Plate Factor times highest dilution factor.

Examples (Underlined Counts Were Used in Determining Report):

Example	1.0 mD	0.1 mL	Ratio	Rule	Report
1	<u>175</u> / <u>208</u>	16 / 17		Α	190 CFU/mL
2	230 / 246	<u>30</u> / <u>36</u>	1.4	В	280 CFU/mL
3	138/162	42 / 30	2.4	В	150 CFU/mL
4	<u>310</u> / <u>330</u>	23 / 19		С	320 Est. CFU/mL

Example	1.0 mL	. 0.1 mL	Ratio	Rule	Report
5	<u>18 / 14</u>	2/0		D	16 Est. CFU/mL
6	<u>0</u> / <u>0</u>	0/0		E	< 1 Est. CFU/mL
7	<u>315</u> / <u>285</u>	27 / 25		F	300 CFU/mL
8	<u>275</u> / <u>320</u>	<u>26</u> / <u>33</u>		G	300 CFU/mL
9	<u>310 / 290</u>	<u>34</u> / <u>36</u>		Н	330 CFU/mL
10	<u>240 / 270</u>	<u>30</u> / Spdr.		Н	270 CFU/mL
11	<u>263</u> / <u>284</u>	L.A. / <u>30</u>		Н	280 CFU/mL
12	TNTC / TNTC	1425 Est. <sup>1</sup> / 1311 Est. <sup>2</sup>		J	14,000 Est.3 CFU/mL

<sup>&</sup>lt;sup>1</sup>Based on an average of 25 CFU/cm<sup>2</sup> when 4 cm<sup>2</sup> were counted

- 5. Record the computed count on the work sheet, but do not record the result on the Final Report Form.
- 6. A second analyst must verify and initial the Computed Count on the work sheet.

  The second analyst must record the result on the Final Report From.

### VI. Quality Control -

- 1. Air Quality Plate, "T-1", must contain ≤15 CFU's. If the Air Quality Plate exceeds 15 CFU's, then the Sample Results are to be recorded as "Laboratory Accident" and a new sample is to be requested.
- 2. Agar Sterility Plates, "Pipet" Plate and "H2O" Plate must not show signs of contamination (excessive growth to be determined by Supervisor). If control plates are contaminated, then the sample results are to be recorded as "Laboratory Accident" and a new sample is requested.
- 3. Each month, each analyst is to count the same plate (a plate in the 30 to 300 CFU range). Counts are compared statistically using the Rp X Sm Method.

<sup>&</sup>lt;sup>2</sup> Based on an average of 23 CFU/cm<sup>2</sup> when 4 cm<sup>2</sup> were counted

<sup>&</sup>lt;sup>3</sup> Based on a Plate Factor of 57 cm<sup>2</sup>

Environmental Microbiology SOP / QA Manual Procedure: HPC Rev. 11/18/98

## Attachment #1

# **Heterotrophic Plate Count Bench Sheet**

(These sheets are found in the Colilert Book - The HPC samples are listed on a separate sheet before the days run of Colilert samples.)

HPC

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# Multi Tube Fermentation (100 mL)

I. Introduction -

Multi Tube Fermentation is the standard test used by the laboratory for detecting total coliform and fecal coliform bacteria in drinking water compliance samples. The historical definition for the coliform group of bacteria has been based on the method used for detection. When using the fermentation test, the coliform group is defined all facultative anaerobic, gram-negative, bacteria as non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hours at 35°C. For drinking water compliance samples the laboratory uses a single 100 mL sample portions and because of the potential problems associated with gas bubbles in large inverted tubes, the gas vials are replaced with bromcresol purple (0.01 g/L). The test consists of two phases presumptive and confirmation and can take anywhere from 48 to 96 hours for completion.

### II. Sample Requirements -

- 1. Maximum allowable elapsed time between sample collection and sample analysis is thirty (30) hours.
- 2. Reject samples for any of the following reasons:
  - A. Sample exceeds 30 hours.
  - B. Information on the Water Bacteriological Report Form (EM-1) is insufficient. (No date or time of collection)
  - C. Insufficient Sample Volume. (< 97.5 mL)
  - D. Sample contains residual chlorine.
  - E. Insufficient air space to facilitate mixing of sample.
  - F. Sample container was not furnished by the Office of Laboratory Services.

# III. Sample Types -

- 1. Drinking water compliance samples only.
- 2. Back-up method for all other drinking water samples and pools.

# IV. Reagents and Equipment -

Reagents

- 1. Lauryl Tryptose Broth (double strength with 0.01 g/L Bromcresol Purple). Prepared by the Media Preparation Section in 250 mL screw cap culture bottles (Corning or Wheaton) and stored in the dark in the cabinets in the water room for no longer than three months at room temperature (<30°C).
- 2. Brilliant Green Bile Broth. Prepared by the Media Preparation Section in 20 x 150 mm screw cap culture tubes and stored in the dark in the cabinets in the water room for no longer than three months (loose lid tubes stored no longer than 2 weeks) at room temperature (<30°C).
- 3. EC Medium. Prepared by the Media Preparation Section in 20 x 150 mm screw cap culture tubes and stored in the dark in the cabinets in the water room for no longer than three months (loose lid tubes stored no longer than 2 weeks) at room temperature.

### Equipment

- 1.  $35.0 \pm 0.5$ °C Incubator. (Walk-In or Environette)
- Sterile Cotton Swabs.
- Metal Racks and Baskets.
- 4. Culture Tube Racks.
- 5. Wax "Chicken" Buckets.
- 6. Tare Bottle with 100.0 ± 2.5 mL range indicated.

### For Quality Control

- 1. 10<sup>-8</sup> Stock of *E. coli*
- 2. Slant of non-lactose fermentating *E. coli*
- 3. (3) 100 mL Sterile Water Samples.
- 4. Inoculating Loops

### V. Procedure

Note: All data for the presumptive and confirmation phase is to be recorded on the MTF work sheet (Attachment #1) in the MTF Records Records Book.

### Presumptive Phase

1. Shake sample 25 times in 7 seconds with a 1 foot movement and pour off excess so that only  $100 \pm 2.5$  mL remains. (Use tare bottle.)

- 2. Pour 100 mL of sample into culture bottle containing 100 mL of double strength lauryl tryptose broth containing bromcresol purple.
- 3. Place inoculated culture bottle(s) into metal rack (holds 30 samples) or metal basket (holds 6-7 samples) and place in a 35.0 ± 0.5°C incubator (Walk-In or Environette) for 48 ± 3 hours on the 24 hour shelf.
- 4. Check cultures in 24 ± 2 hours. If culture(s) are clear purple (negative) or cloudy purple (Turbid), move to the 48 hour shelf. If culture(s) are yellow (Presumptive Positive), remove from the incubator and obtain the corresponding Water Bacteriological Report Form (EM-1). Record a "+1" in the "p/a24" column. Place the corresponding Water Bacteriological Report Form, EM-1 into the 24 Hour BG box. The sample is now ready for the Confirmation Phase.
- 5. After 48 ± 3 hours of incubation, remove remaining cultures from the incubator. Cultures that are clear purple are negative for total coliform bacteria. For the negative cultures, record the date they are read in the "rpt date" column and the analysts initial's in the "init" column. Also, record the time read out to the side of the last column. Note, the report date and initials can be recorded in the top of the column and lines drawn down. The sample is now ready for reporting.

If a culture is cloudy purple (turbid), set it aside, locate the corresponding EM-1 form and place it in the 24 Hour BG box and record a "T" in the "P/A48" column. The sample is now ready for the Confirmation Phase.

If a culture is yellow, set it aside, locate the corresponding EM-1 form and place it in the 24 Hour BG box and record a "+1" in the "P/A48" column. The sample is now ready for the Confirmation Phase.

### Confirmation Phase

6. For each presumptive positive sample (yellow and turbid cultures) submitted for the Confirmation Phase, obtain one tube containing EC Medium (EC) and one tube containing Brilliant Green Bile Broth (BG). Label each tube with the laboratory number as follows: using a wax pencil, label the glass BG tube with the sample number and label metal lid of the EC tube with the laboratory number. (The lid of the EC tube is numbered because the tube is placed in a water bath and if the glass tube is numbered, it may

wash off.)

- 7. Mix the presumptive positive culture by swirling it. Using a sterile swab, dip into the presumptive positive culture and then transfer into EC and then into BG (in that order). Record the time of the transfer in sample log book at the bottom of the appropriate column (p/a24 or P/A48).
- 8. Place the BG tube on the 24 Hour BG shelf in the  $35.0 \pm 0.5$ °C Walk-In Incubator and place the EC tube in the  $44.5 \pm 0.2$ °C Fecal Water Bath.
- 9. After 24 ± 2 hours remove the EC tubes from the Fecal Water Bath and gently swirl to dislodge any gas bubbles. Gas in the inverted gas vial is considered a fecal coliform positive and is to be recored as a "+1" in the "fc data" column. Clear tubes with no gas and turbid tubes with no gas are considered negative for fecal coliform and are to be recorded as "-1" in the "fc data" column. Fecal coliform positive samples are to recorded as "Pres" in the "fecal rp" column.
- 10. After 24 ± 2 hours the BG tubes are to be removed from the 24 Hour BG shelf and examined for gas production. If there is no gas in the inverted vial, record as "-1" in the "conf/c" column and place back into the Walk-In Incubator on the 48 Hour BG shelf for another 24 hours (total time in BG is 48 ± 3 hours). Place corresponding EM-1 form into the 48 Hour BG Box.

If there is gas in the inverted gas vial then the sample is confirmed as total coliform positive. Record a "+1" in the "conf/c" column and "Pres" in the "total" column. Record the report date in the "rpt date" column and analysts initials in the "init" column. Also, record the time to the right of the "init" column. The sample is now ready for reporting.

11. After 48 ± 3 hours, remove all BG tubes from the Walk-In and examine for gas production. If there is gas in the inverted gas vial, then the sample is confirmed as total coliform positive. Record a "+1" in the "conf/48" column and "Pres" in the "total" column. Record the report date in the "rpt date" column and analysts initials in the "init" column. Also, record the time to the right of the "init" column. The sample is now ready for reporting.

If there is no gas in the inverted gas vial, then the sample is considered Invalid. record a "-1" in the "conf/c" column and "Inv" in the "total" column. Record the report date in the "rpt date" column and analysts initials in the "init" column. Also, record the time to the right of the "init" column. The

sample is now ready for reporting.

### Reporting

12. After analysis is complete and the data is recorded in the log book, the corresponding EM-1 forms must be completed. For samples that are negative for total coliform, record an "X" in the in the "Total Coliform Absent" Box on the EM-1 form.

For samples that are total coliform positive, record and "X" in the "Total Coliform Present" Box on the EM-1 form. Then record the fecal coliform results by placing an "X" in the appropriate "Fecal Coliform" Box (either Present or Absent). If a sample is "Present" for total coliform, then there must be a result for fecal coliform.

For invalid samples, those that did not produce gas within  $48 \pm 3$  hours in Brilliant Green Bile broth, place an "X" in the "Invalid" box, an "X" in the "Turbid" box and an "X" by the "Send Replacement Sample"

- 13. After all EM-1 forms are marked, they are to be placed in the "To Be Checked" Basket.
- 14. A Microbiologist II or higher then will check the EM-1 forms against the log book for precision and accuracy and will initial all total coliform positive results in the log book to the right of the last column.
- 15. If a sample is submitted for compliance with the Safe Drinking Water Act (SDWA) and is positive for total coliforms, the EM-1 form is pulled by the analyst checking the forms and marked with a "post-it" note indicating that it is to be faxed to the Office of Environmental Health Services Environmental Engineering Division. These forms are immediately faxed by the staff of the General Reporting Office.
- 16. All EM-1 forms are then taken to the General Reporting Office where they are sorted, faxed, mailed and stored.

# VI. Quality Control

Each batch of laboratory prepared media must be checked before use with positive and negative controls.

- When media is delivered from the Media & Glassware Preparation Unit, pull 4 samples from each batch (double strength lauryl tryptose broth, brilliant green bile broth and EC Medium).
- 2. Label as follows: 1 bottle/tube "-" (Negative Control), 1 bottle/tube "NLF" (non-lactose fermenting E. coli) and 2 bottles/tubes "+" (Positive Control E. coli).

For 100 mL Double Strength Lauryl Tryptose Broth:

- 3. Obtain four 99 mL dilution blanks per batch. Label as in Step #2. Add nothing to the "-" dilution blank, add one loopful of NLF (from a slant) to the dilution blank labeled "NLF" and add 0.5 mL of 10<sup>-8</sup> stock of *E. coli* to each dilution blank labeled "+".
- 4. Shake each dilution blank and add to the appropriate labeled culture bottle and incubate as outlined in the procedure above above.
- 5. Record Results on QC Form (Attechment #2).

For Brilliant Green Bile Broth and EC Medium:

- 6. Add 0.5 mL of 10-8 stock culture of E. coli to tubes labeled "+", add a loopful of non-lactose fermentating *E. coli* to the tube labeled "NLF" and add nothing to the tube labeled "-".
- 7. Incubate as outlined in the procedure in Section V above.
- 8. Record results on QC Form (Attachment #2).

Environmental Microbiology SOP / QA Manual Procedure: MTF 100 mL Rev. 4/19/00 (Changes 6/13/00)

Attachment #1
MTF Bench Sheet

# Attachment #2 Media Productivity and Sterility Checks

# I. Equipment and Reagents

Equipment	Manufacturer	Model Number	Comments
Autoclave	AMSCO (Steris)  American (AMSCO/Steris)  Market Forge	2052 Isothermal 3051 Isothermal "Large" Sterilmatic	Waste and Discards Media and Reagents Laboratory Waste Backup
Balances	American Scientific Products OHAUS Metler	1200P E 400 D AE 240	General Weighing Some Reagents/Wt. Loss Pipet Calibrations
Block Heater	Advanced Instruments Lab-Line	2010A 2053ID	For Fluorophos For SNAP
Colony Counters	American Optical Reichert Sensaur (Darkfield)	3325 3325 Bacti-Counter	Quebec (Darkfield) Quebec (Darkfield)
Conductivity Meter	Fisher	09-226-2	
Culture Dishes	Falcon Falcon Fisher Pyrex Kimax	1029 1007 08-757-13	15X100 mm 15X60 mm 15X100 mm (Back-up) 15X100 mm (Glass) 15X100 mm (Glass)
Culture Tubes/ Bottles	Pyrex Wheaton Fisher Pyrex Pryex Pryex Corning Vacutainer	1395-250 219757 14-962-26G 9820-25 9825-38 9820-10 45060-1075 1370-160 366435	250 mL Bottle 250 mL Bottle 20X150 Screw Cap Tube 20X150 Loose Lid Tube 38X200 Screw Cap Tube 10X75 "Gas Vial" 10X75 "Gas Vial" Dilution Bottles - Milk 16X75 "Inhibitor Tubes"
Electronic Somatic Cell Counter	FOSS Food Technology	Fossomatic 90	
Escher Stoppers	Weber	3026-20	For Milk Dilution Bottles
Filters	Foss Food Technology Grainger	FRP-95-160/(1) 6CZC89 1R424A	Before Dryers Silica (2) on Air Line (Foss 90)
Fluorophos /	Advanced Instruments	FLM 200	
Fluorophos Excitation Filter	Advanced Instruments	FL0330	

West Virginia Department of Health and Human Resources Bureau for Public Health Office of Laboratory Services ENVIRONMENTAL MICROBIOLOGY LABORATORY DRINKING WATER PROGRAM



# STANDARD OPERATING PROCEDURE

# STANDARD METHODS 9223B ENZYME SUBSTRATE COLILERT®/COLILERT18® 100 mL

Revision: 2.0

Author: Thomas L. Ong (2012)

HHR Program Manager – Environmental Microbiology Date

APPROVED:

HHR Program Manager Quality Control Coordinator Date

HHR Laboratory Director III – Office of Laboratory Services Date

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### SM9223B - Enzyme Substrate Test (Colilert/Colilert18 100 mL)

### 1.0 Introduction -

Colilert Reagent is used for the simultaneous detection and conformation of total coliforms and  $E.\ coli$  in water, which is based on the Defined Substrate Technology (DST). DST utilizes indicator-nutrient which cause target microbes contained in the sample and incubated in the DST reagent system to produce a color change (or another signal i.e., fluorescence), both indicating and confirming their presence. Coliforms as defined by this method are all bacteria that possess the enzyme  $\beta$ -D-galactosidase with  $E.\ coli$  also possessing the enzyme  $\beta$ -glucuronidase. The enzyme in Coliform will cleave the Chromegenic Substrate of the Test Reagent releasing the Chromogen just as the enzyme in  $E.\ coli$  will cleave the Fluorogenic Substrate releasing a fluorogen.

Standard Operating Procedure, Revision 2.0 makes the following changes from Revision 1.0:

- Incorporates Colilert18;
- > Adds more reasons for sample rejections;
- Adds "Compliance Samples" as the primary sample type;
- More detailed Equipment and Reagent lists with photographs;
- Updated Analytical Methodology and included photographs;
- Revised Quality Control (Section 6.0) to reflect current practices
- > Added a Reference Section (Section 7.0)

### 2.0 Sample Requirements-

- 2.1 For Compliance Samples and Bottled Waters: Maximum allowable elapsed time between sample collection and sample analysis is thirty (30) hours.
- 2.2 For Non-Compliance: Maximum allowable elapsed time between sample collection and sample analysis is forty eight (48) hours.
- 2.3 Reject samples for any of the following reasons:
  2.3.1 Insufficient air space to facilitate mixing of sample.
  2.3.2 Sample contains residual chlorine. (Blue flash appears)
  - 2.3.3 Sample exceeds maximum allowable time requirements.
  - 2.3.4 Information on the Water Bacteriological Report Form (EM-1) is insufficient. (No date or time of collection)
  - 2.3.5 Sample container was not furnished by the Office of Laboratory

### Services.

- 2.3.6 Insufficient Sample Volume. (< 100.0 mL)
- 2.3.7 Invalid Date of Collection
- 2.3.8 Unauthorized Collector
- 2.3.9 Delinquent Account

### 3.0 Sample Types\* -

- 3.1 Compliance Samples
- 3.2 Repeat/Replacement Samples
- 3.3 Special Purpose Samples
- 3.4 Private Samples
- 3.5 Home Loans
- 3.6 Bottled Waters
- 3.8 Swimming Pools
- 3.9 Flood/Disaster Samples
  - \*Samples received before 12:00 p.m. are analyzed with Colilert. Samples received after 12:00 p.m. are analyzed using Colilert18

# 4.0 Reagents and Equipment -

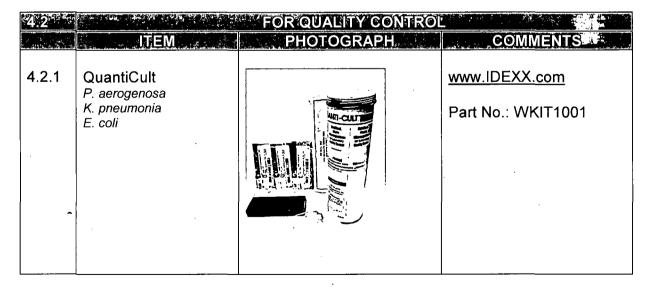
4.1	ITEM	FOR ANALYSIS PHOTOGRAPH	COMMENTS
4.1.1	35.0°C Incubator (Walk-In or Environette)		Built In The Water Room. For Maintenance Issues, contact OLS Maintenance Department.
4.1.2	Long Wavelength 366/365 nm Ultraviolet Lamp, 6-watt		www.IDEXX.com  Part No.: WL160  Replacement Bulb: Part No.: WL161  Black-Ray UVL-56 currently in use
4.1.3	Color/Fluorescence Comparator		www.IDEXX.com Part No.: WP104
4.1.4	Sample Bottle (120 mL) 120 mL, Tear-Off Label with Sodium Thiosulfate	Bu # 162 ***Odo! Der 4/00/1	www.IDEXX.com Part No.: WV120ST-200

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4124	AUEMS.	FOR ANALYSIS AND PROPERTY OF THE PROPERTY OF T	COMMENTS
4.1.5	Sample Volume Comparator	100.0 mL	Prepared In-house using a Class A Graduated Cylinder.
4.1.6	Colilert Reagent	collect Collect	www.IDEXX.com Part No.: WP200I
4.1.7	Colilert18 Reagent	Collect St.	www.IDEXX.com Part No.: WP200I-18
4.1.8	Ethanol 70%	Airow	Prepared in-house by diluting 190 Proof Ethanol (available from OLS Stock Room) with Distilled Water. 700 mL EtOH + 250 mL Dist. Water

4.1		FOR ANALYSIS	
100	ITEM	PHOTOGRAPH	COMMENTS
4.1.9	Covered Water Bath 44.5°C		Precision Covered Water Bath  Part No.: 51221035
4.1.10	Masking Tape		Available from the Office of Laboratory Service's Stock Room
4.1.11	Sharpie	Shappe	Available from the Office of Laboratory Service's Stock Room

<b>.4</b> *1*/*/	TEM	FOR ANALYSIS PHOTOGRAPH	COMMENTS
4.1.12	Timer	VWR 62	www.VWRSP.com Part No.: 89087-398
4.1.13	Metal Rack / Basket		www.VWRSP.com 10"x6'x6" Aluminum Basket Part No.: 60905-127 Larger Baskets are from left over Glassware Washer Parts. Racks also available from IDEXX.com

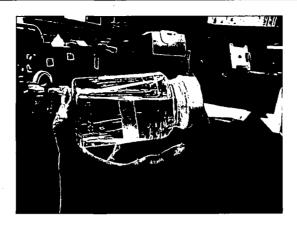


4.2		FOR QUALITY CONTROL	
	, ITEM	PHOTOGRAPH	COMMENTS
4.2.2	Graduated Cylinder 100 mL, Class A		Kimax Part No.: 20026 or equivalent
4.2.3	Tryptic Soy Broth, 25 mL 1X Strength		Ordered from and prepared by the Media & Glassware Preparation Section Using BD Tryptic Soy Broth.
4.2.4	Lexmark T640 Laser Printer	O. C.	For problems and replacement, contact IT.

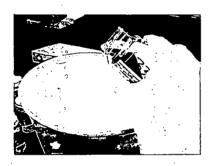
4.2		FOR QUALITY CONTROL PHOTOGRAPH	COMMENTS
4.2.5	Avery #5167 Labels	Easy Peel White Mailing Labels  Laser  2267	www.OfficeMax.com Part No.: 07100442
4.2.6	pH Meter		Orion 920A with 900A Printer

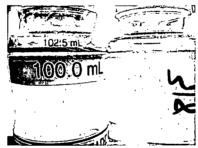
## 5.0 Analytical Methodology -

- 5.1 Test Procedure: Colilert Reagent is used for all samples received and analyzed before 12:00 p.m. unless a more rapid turnaround is required. All samples received and analyzed between 12:00 p.m. and 5:00 p.m. are analyzed with Colilert18.
  - 5.1.1 Sanitize area with 70% Ethanol and wash hands.
  - 5.1.2 Shake sample 25 times within seven (7) seconds with a one (1) foot movement.



5.1.4 Remove the lid (do not lay the lid down – and pour off sample into a wax bucket down to the 100 mL line (use sample volume comparator, 100.0 to 102.5 mL), set lid back on test bottle. If too much sample is removed and the remaining sample volume is < 100.0 mL, the sample must be rejected and listed as a "Laboratory Accident". Raise the lid and aseptically add a packet of the appropriate Colilert reagent (i.e., Colilert / Colilert18).





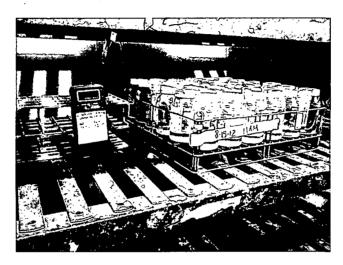


- 5.1.5 Shake samples until Colilert reagent dissolves.
  - 5.1.5.1 Colilert18 Only: Place samples in the 44.5°C water bath, cover and set countdown timer for 7 minutes.



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5.1.6 Place all samples in either a metal basket or metal rack, label the racks or baskets with the Date, Time and 18 Hr (if Colilert18) using masking tape and sharpie, then place in Walk-In Incubator on shelves that contain digital thermometers. Environette may be used in case of mechanical problems with the Walk-In Incubator.



5.1.7 Incubate samples for 24 to 28 hours (Colilert18 Only: 18 to 22 hours) at 35.0±0.5°C

### 5.2 Test Results

5.2.1 Remove samples from the incubator after 24 to 28 hours of incubation (18 to 22 hours for Colilert18). Samples must be removed from the incubator within 28 hours (22 hours for Colilert18).

### Ex. 5 - Deliberative



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5.2.2 Examine samples for the presence of a yellow color (confirming the presence of coliform bacteria) that is equal to or greater than the comparator. Samples that are slightly yellow, but not as yellow as the comparator, must be place back into the incubator to incubate for the full 28 hours. Samples left in the incubator for more than 28 hours (22 hours for Colilert18) must be reported as "Laboratory Accident" unless they are clear.

Ex. 5 - Deliberative



Ex. 5 - Deliberative

Ex. 5 - Deliberative

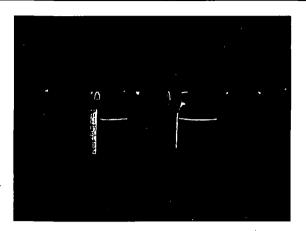
- 5.2.3 If a sample has a yellow color equal to or greater than the comparator, then a +1 is to be recorded in the "Conf24" column and a "Pres" in the "Total Coliform" column of the colilert bench sheet. The sample is considered "Total Coliform Positive".
- 5.2.4 All Yellow Samples (Total Coliform Positive Samples) must be taken into the Walk-In Incubator and with the door shut and lights turned off, checked for fluorescence with the 366 or 365 nm UV light. Samples with fluoresce equal to or greater than the comparator are Positive for E. coli and must be marked on the lab number label with a pen or marker. The samples that fluoresce must then be marked with a +1 in the first "E. coli" column and a "Pres" in the second "E. coli" column on the colilert bench sheet. If the sample did not fluoresce, then it must then be marked with a -1 in the first "E. coli" column and an "Abs" in the second "E. coli" column on the colilert bench sheet.

Ex. 5 - Deliberative

Ex. 5 - Deliberative

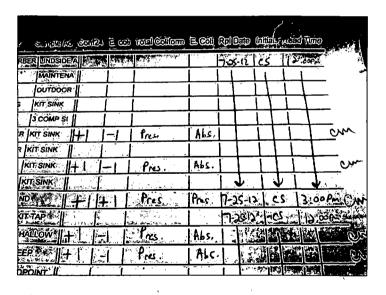
Ex. 5 - Deliberative

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5.2.5 If a sample is clear (Negative for Total Coliforms) then record on the bench sheet the date that the sample analysis was completed in the "Rpt Date" column and the analysts initials (analyst reading the results) in the "Initials" column.

Note: The report date and analysts initials may be recorded on the top line and then arrows drawn down.



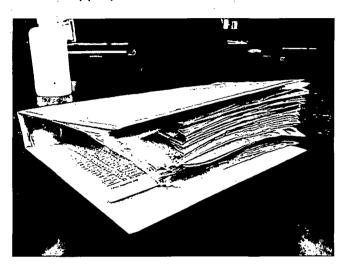
5.2.6 After the data has been entered on the bench sheet for a particular sample then the Water Bacteriological Report Form (EM-1) is to be completed. Total Coliforms are to be marked as "Present" or "Absent". E. coli only has to be marked as "Present" or "Absent" if

Ex. 5 - Deliberative

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Total Coliforms are Present.

5.2.7 After all EM-1 forms are marked, they are to be placed in Log Book with the appropriate Bench Sheets.



5.2.8 All forms are to be checked by a Microbiologist II or higher. All samples with Total Coliform Positive Results must be initialed by the analyst checking them on the bench sheet. Initials are to be placed to the right of the initials of the analyst reading the test.

### 5.3 Sample Disposal

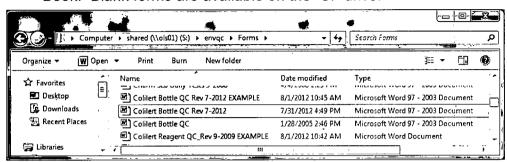
- 5.3.1 All samples that are Total Coliform Absent (clear) are poured down the sink and the sample bottles are disposed of in the hard trash.
- 5.3.2 All samples that are Total Coliform Positive and/or E. coli Positive are taken to the Media & Glassware Preparation Section for autoclaving (132°C for 45 minutes). Once samples have been autoclaved, the contents can be poured down the sink and the sample bottles disposed of in the hard trash.

### 6.0 Quality Control

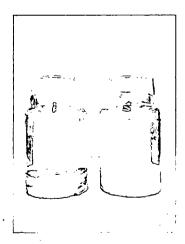
- 6.1 Sample Bottles -
  - 6.1.1 Each lot of Colilert Test Bottles received must be checked using the

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"Quality Control – Colilert Bottles" Form in the Water Quality Control Book. Blank forms are available on the "S:" drive:



- 6.1.2 Record the "Date of Check" the "Lot Number" and the "Expiration Date" on the "Quality Control Colilert Bottles" Form in the Water QC Book.
- 6.1.3 Check three (3) bottles from each lot for sterility by adding 25 mL of Tryptic Soy Broth to each bottle, incubate at 35.0 ± 0.5°C for 48±3 hours. After the 24 hours, examine bottles for signs of growth (Turbidity as indicated by the bottle on the right in the photograph below) and incubate another 24 hours. Record results in the "Sterility" Column as "Number of Bottles", "A" (Absent-No-growth) or "P" (Positive Turbid). If any bottles test Positive, recheck three (3) more bottles. If any bottles on the recheck test positive, contact the Supervisor.



6.1.4 Verify the 100 mL mark on one (1) bottle from each lot by filling the

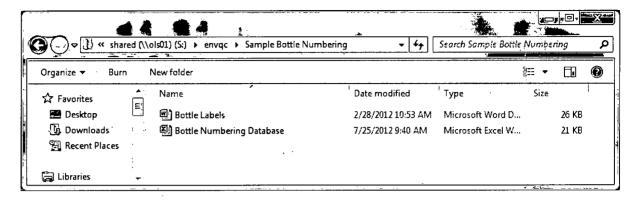
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bottle to the 100 mL mark with water then pour into a Class A graduated cylinder. Record the actual volume in mL under the "100 mL Verification Column"; if the volume reads outside of the 100.0 - 102.5 mL range, the 100 mL mark cannot be used to measure the sample volume.

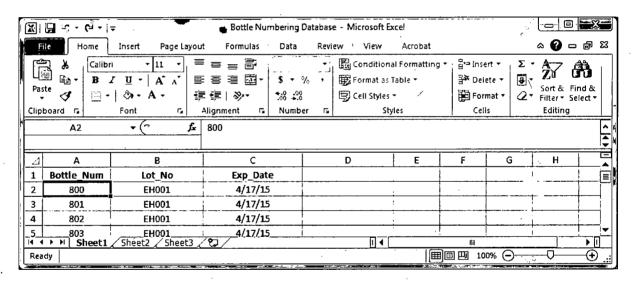
- 6.1.5 Check one (1) bottle from each lot (can be the same bottle that is used for the volume check) for autofluorescence by examining the bottle in the dark in the Walk-In Incubator with the 366 nm UV light. Place a "NO" in the "Autofluorescence" column if the bottle does not fluoresce and an "YES" if it fluoresces. If the bottle fluoresces, then check another from the same lot. If the recheck fluoresces, contact the Supervisor.
- 6.1.6 Record "Analysts Initials" and "Comments" (if any).

COLILERT BOTTLES — QUALITY CONTROL  1. STERILITY: Add 25 mLs of Tryptic Soy Broth to each of 3 Bottles and incubate for 48±3 hours @ 35.0°±0.5°C.  Check for growth in 24 and 48 hrs. Report No Growth as "(# of Bottles) A". Report Growth as "(# of Bottles) P".  If any show growth, repeat test. If repeat test shows growth reject lot and contact manufacturer.  2. AUTOFLUORESCENCE: Check 1 bottle with a 6 watt, 366 nm UV light. Record Autofluorescence as "NO" or "YES". If "YES", contact manufacturer.  3. 100 mL VERIFICATION: Fill bottle to 100 mL line with water. Measure volume with a Graduated Cylinder and record Volume.									
DATE REC'D	LOT # Exp. Date	DATE EXAM'D	STER "#P" O 24 Hr		AUTO- FLUORESCENCE	100 mL VERIFICATION	COMMENTS	ANALYST INITIALS	
7-13-12									
					·				

6.1.7 Once a Lot of Sample bottles Passes Quality Control, they are labeled with a "Bottle Number", "Lot Number" (also stamped on bottom of bottle) and an "Expiration Date" using the Bottle Numbering Database (MS Excel) and Bottle Labels (MS Word) located in the Sample Bottle Numbering Folder in "envqc" on the shared drive – shared(\\ols01)(S:).

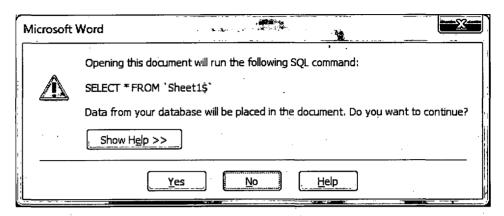


6.1.8 Open the *Bottle Number Database* and enter a Starting Bottle Number in Cell A2 (last number used is located in Cell A401) and press "Enter"; enter the Lot Number in Cell B2 and press "Enter"; enter the Expiration Date in Cell C2 and press "Enter"; Save and then Close.

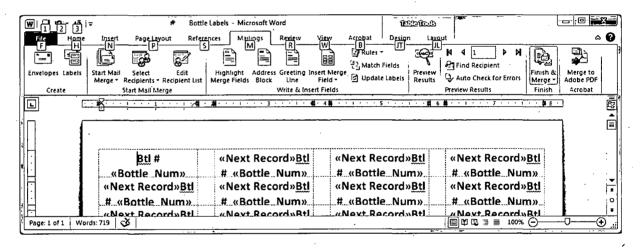


6.1.9 Open the *Bottle Labels* document. Click "YES" when the following prompt is displayed:

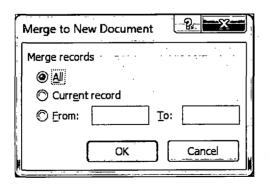
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6.1.10 Select the "Mailings" tab and then click "Finish & Merge", "Edit Individual Documents..."

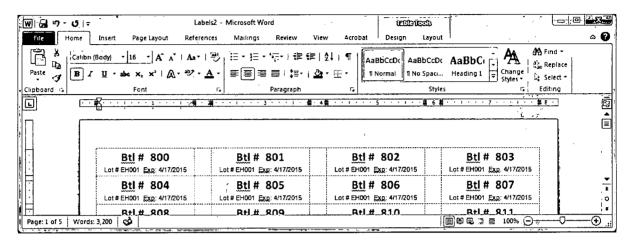


6.1.11 Make sure "All" is selected and Click "OK" at the following prompt:

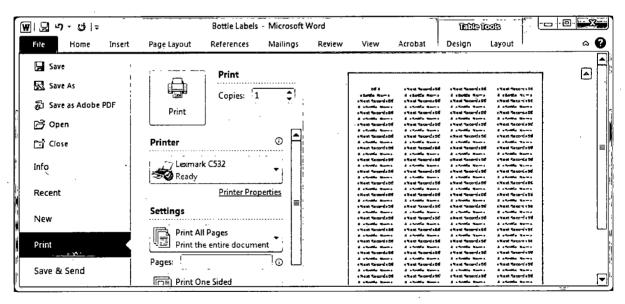


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6.1.12 A new document will be created (Labels2) with 400 labels.



6.1.13 Load the Upper drawer of the Laser Printer (Lexmark T640) with blank labels, Avery #5167 or equivalent, (place labels face down – red Avery backing facing up) select "File" and "Print".

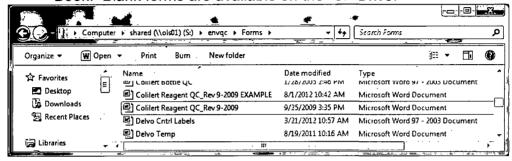


- 6.1.14 Close all windows and when prompted, select "Don't Save".
- 6.1.15 Place labels on sample bottles and deliver to the Container Room for shipment to clients.

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## 6.2 For Colilert Reagent -

6.2.1 Each lot of Colilert Reagent must be checked for Autofluorescence, pH Verification, Sterility and Productivity before use using the "Colilert Reagent – Quality Control" form located in the Water Quality Control Book. Blank forms are available on the "S:" Drive:



- 6.2.2 Record "Date Examined", "Date Received", "Type" (24 or 18 Hours), "Lot Number" and "Expiration Date" on "Colliert Reagent Quality Control" Form.
- 6.2.3 To check for Autofluorescence:
  - 6.2.3.1 Aseptically add one (1) packet of colliert reagent to 100 mL of sterile water in a colliert test bottle and shake to dissolve completely.
  - 6.2.3.2 Check for fluorescence in a dark room (Walk-In Incubator with lights off and door shut) with a 6 watt, 366 nm UV light.
  - 6.2.3.3 If no fluorescence is observed, record "NO" in the "Autofluorescence" Column. If fluorescence is observed, record "YES" and notify Section Supervisor and Manufacturer.
- 6.2.4 For pH Verification:
  - 6.2.4.1 Take the sterile water and reagent mixture used for the Autofluorescence check to the Media & Glassware Section and check the pH with the Orion pH Meter

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(must be calibrated just prior to use – refer to SOP: *Quality Control: Water Program* for pH meter calibration protocol).

- 6.2.4.2 Record the pH in the "pH' column and tape the pH meter printout in the Environmental Microbiology Media Prep Log Book (in the Media & Glassware Section)
- 6.2.4.3 pH must read 7.3 ± 0.1. If pH falls outside of appropriate range, notify Section Supervisor and Manufacturer.

## 6.2.5 For Reagent Productivity:

6.2.5.1 Remove a Quanti-Cult Set (consisting of 3 Rehydration Fluid Vials and 3 Organism Vials – *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia* coli) from the 7-11 Refrigerator. Pre-warm the Rehydration Vials (blue caps) in the 35.0°±0.5°C incubator for 10 to 15 minutes.



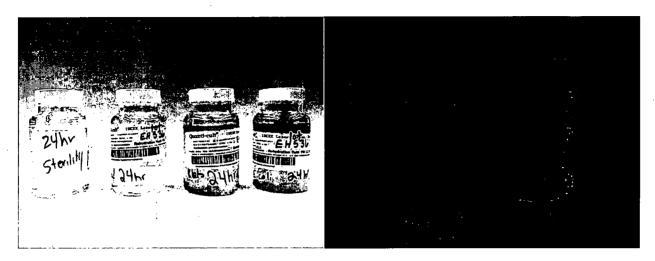
- 6.2.5.2 Pre-warm 4 sample bottles containing 100 mL of sterile water and labeled with the organism name (can use the pre-made labels off of the foil packets included with the Quanti-Cult) at 35.0°C±0.5°C for 25 30 minutes. One bottle for each of the 3 organisms plus a 4<sup>th</sup> for a sterility control.
- 6.2.5.3 Remove the rehydration vials from the incubator and discard the blue cap on the rehydration vial and replace with a cap from the organism vial. Discard vial

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	containing desiccant into autoclave waste receptacle.
6.2.5.4	Insert the rehydration vial (with the organism cap) into the included foam rack and repeat for the remaining two organisms.
6.2.5.5	Invert the foam rack and incubate at 35.0°±0.5°C for 10 minutes.
6.2.5.6	Remove vial from rack and tap cap gently to mix.
6.2.5.7	Remove the cap and verify that there are no black particles remaining in the cap. If black particles remain in the cap, re-incubate for another 10 minutes.
6.2.5.8	Pour the contents of the vial containing the organism into the appropriately labeled, pre-warmed sample bottle containing 100 mL of sterile water. Repeat for the remaining two organism vials.
6.2.5.9	Add one packet of Colilert (or Colilert18) Reagent to each of the 4 Sample Bottles, shake to dissolve completely and incubate and 35.0°±0.5°C for the appropriate time (24 to 28 hours for Colilert, 18 to 22 hours for Colilert18).
6.2.5.10	After the appropriate incubation period, record the results under "Reagent Productivity". Use "-" to indicate No Yellow Color or Fluorescence and a "+" to indicate the Presence of a Yellow Color or Fluorescence. Any results other than those listed below must be immediately reported to the Section Supervisor:

:::Stei	erility P. aeruginosa K. pneu		umonia	E. coli			
Yellow	Fluorescence	Yellow	Fluorescence	Yellow	Fluorescence	Yellow	Fluorescence
•	-	•	-	+	-	+	+



6.2.6 Record Comments (if any) and initials in the "Analyst" column when recording the results.

2. pH: Us 0. 3. REAGE m	"YES", Do Ning the reage I, Do Not Use NT PRODU L Sterile Wat	Not Use and contains and sterile and contact CTIVITY: Iter & Reagen	ontact manufa water mixtur manufacturer noculate 3 sep n) at 35.0±0.5	agent to 100 m acturer. re in #1 above. r. arate Colilert °C for 24-28 h	LERT REAG L Sterile Water in a C check and record pH Bottles (100 mL Steri rs for Colilert and 18- gractions as indicated	to the ne te Water 22 hrs fur	ssel and arest 0,1 & Reage r Colitert	check w units us int in each	ith a 6 w ing the c :h) with cord "+"	att, 366 alibrate Organis for Yell	nm UVI d pH me ms listed low Colo	ter in th I in table or and FI	ie Media s. Incub luorescei	Section. ate all 3 ace OR	If pH does not e plus a Sterility Co for No Color C	qual 7.3 ± ontrol (100 Change and
DATE EXAM'D	DATE REC'D	TYPE 18/ 24	LOT#	EXP. DATE	AUTO- FLUORESCENCE	pН	Ster Yellow	ility Fluor	P. aeru Yellow		ODUCTI K. pnet Yellow +		E. ·	coli Fluor +	COMMENTS	ANALYST INITIALS
7-26-12	7-13-12	24	EH536	5-26-13	NO	7.2	-	-		-	+	-	+	+		TLO
7-26-12	7;13-12	18	E526	8-17-13	NO	7.3	-	-	-	-	+	-	+	+		TLO

## 7.0 Safety

- 7.1 The following Material Safety Data Sheets are on file in the Water Room MSDS Book and on the "S:" Drive:
  - Colilert
  - ➤ Colilert18
  - Colilert Comparator
  - Quanti Cult
  - BD Tryptic Soy Broth
  - Colilert Sample Vessels (Sample Bottles)

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167 - 11th Avenue, South Charleston, WV 25303 Phone: 304-558-3530 Fax: 304-558-2006

## 8.0 References

- 8.1 EPA 815-R-05-004, *Manual for the Certification of Laboratories Analyzing Drinking Water*, 5<sup>th</sup> Edition, January 2005, Environmental Protection Agency.
- 8.2 American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, and Water Environment Federation. 20<sup>th</sup> Edition, Washington, D.C.
- 8.3 IDEXX 06-12999-04, Colilert (Package Insert), 2011, IDEXX Laboratories, Inc.
- 8.4 IDEXX 06-02027-18, Colilert-18 (Package Insert), 2011, IDEXX Laboratories, Inc.
- 8.5 IDEXX 06-01964-02, Quanti-Cult (Package Insert), IDEXX Laboratories, Inc.

ELEMENT  1. PERSONNEL  Supervisor/Consultant  Does the supervisor of the microbiology laboratory have a bachelor's degree in microbiology, blology, or equivalent?  Has a supervisor with a degree in a subject other than those listed above had at least one college-level microbiology aboratory course in which environmental microbiology was covered?  In addition, has the supervisor had a minimum of two weeks training at a Federal or State agency or academic institution in microbiological analysis of drinking water or 80 hours of on-the-job training in water microbiology at a certified laboratory, or other training acceptable to the State or EPA?  If a supervisor is not available, and a waiver has not been granted as per Section 3.3, is a consultant with the same qualifications substituted?  If a supervisor is not available, and a waiver has not been granted as per Section 2.3, is a consultant with the same qualifications substituted?  If a supervisor is not available, and a waiver has not been granted as per Section 3.3, is a consultant with the same qualifications substituted?  Can the laboratory supervisor demonstrate that all laboratory personnel have the ability to satisfactorily perform the analyses to which they are assigned?  Can the laboratory supervisor demonstrate that all data reported by the laboratory meets the required quality assurance and regulatory criteria?  Analyst (or equivalent job title)  Does the analyst have at least a high school education, a minimum of three months bench experience in water, milk or food microbiology, training in microbiological analysis of drinking water acceptable to the State (or EPA), and a minimum of 30 days on-the-job training under an experienced analyst?  Has the analyst demonstrated acceptable results on unknown samples before analyzing compliance samples?  Waiver of Kademic Training  Has the certification authority waived the need for the above specified training for supervisors of laboratories associated with drinking water systems that only analyze samples from
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LABORATORY FACILITIES  Does the laboratory have facilities that are clean and temperature and humidity
Does the laboratory have facilities that are clean and temperature and humidity
controlled, and with adequate lighting at the bench tops?
The state of the s
Does the laboratory maintain effective separation of incompatible testing areas?
Does the laboratory control access where appropriate, and minimize traffic flow
through the work areas?
Does the laboratory ensure that contamination does not adversely affect data
Quality?
Does the Jahoratony have bench tone and floors that are easily cleaned and
disinfected?
Does the laboratory have sufficient space for processing samples; storage space
for media glassware and nortable equipment floor space for stationary
equipment; and areas for cleaning glassware and sterilizing materials?
The state of the s
Does the laboratory have provisions for disposal of microbiological wastes?
3. LABORATORY EQUIPMENT AND SUPPLIES
Does the laboratory have the equipment and supplies needed to perform the
approved methods for which certification has been requested?
pH meter 3.1
pH meter     3.1       Are accuracy and scale graduations within ±0.1 units?     3.1.1       Are pH buffer aliquots used only once?     3.1.2       Y

January 18-19, 2012

ELEMENT	ITEM	Y/N/O	COMMENTS
Are electrodes maintained according to the manufacturer's recommendations?			
	3.1.3	Y	
QC Are pH meters standardized before each use period with pH 7.0 and either 4.0 or 10.0 standard buffers, whichever covers the desired pH of the media or		Y	
QC Are both the date and buffers used recorded in a logbook along with the analyst's initials?		Y	
QC Is the pH slope recorded monthly, after calibration?	3.1.5	Y	
QC If the pH meter does not have a feature to automatically calculate the slope,	3.7.0		
but can provide in the pH in millivolts, is the formula in Section 3.1.5.1 used to	3 1 5 2	0	
calculate the slope?	~ ` ·		
QC If the slope is below 95% or above 105%, are the manufacturer's instructions followed for meter or electrode maintenance and general cleaning?	3.1.6	Y	
QC Are commercial pH buffer solutions dated when received and when opened?		Y	
QC Are pH buffer solutions discarded by the expiration date?		Υ	
Balance (top loader or pan)	3.2		
Does the halance have a readability of 0.1 d2	3 2 1	Υ	
Does the balance have a sensitivity of at least 0.1 g for a load of 150 g, and 1 mg			
plot a load of 10 g of less?		Υ	
QC Are the balances calibrated monthly using ASTM Class 1, 2, or 3 weights	3.2.3	Y	
(minimum 3 traceable weights which bracket laboratory weighing needs, with a QC Are non-reference weights calibrated every six months with reference		0	
QC Are calibrations recorded in a logbook with the initials of the individual			
performing the calibration?		Υ	
QC Are correction values on file and used?		Υ	
QC Are reference weights re-certified every five years?		Υ	
QC Are damaged or corroded weights replaced?		Y	
QC Are service contracts or internal maintenance protocols and maintenance records available?	3.2.4	Y	
QC Is maintenance, calibration, and cleaning conducted at least annually by a			
qualified independent technician, unless the need is modified or waived by the		Υ	
Temperature Monitoring Device	3.3		•
Are glass, dial, or electronic thermometers graduated in 0.5°C increments (0.2°C			
increments for tests which are incubated at 44.5°C) or less, except as noted for		Υ	
hot air ovens (Section 3.6.1) and refrigerators (Section 3.9.1)?			
Does observation of glass thermometers indicate no separation in fluid columns?	-	Υ	
Are only dial thermometers which can be edirected used?		0	
Are only dial thermometers which can be adjusted used?		0	
QC Are glass and electronic thermometers calibrated annually and dial			·
thermometers quarterly at the temperature used, against a NIST-traceable reference thermometer or one that meets the requirements of NBS Monograph	3.3.2	Y	
SP 250-23?			BATHS NEED DONE JAN 2012
QC Are both the calibration factor and calibration date indicated on the		Y	BATTIS NEED BOINE SAIN 2012
		I	
Is the following calibration information recorded in a QC record book?     Serial number of the laboratory thermometer		Υ	
- Serial number of the NIST-traceable thermometer (or other reference		Y	
thermometer)			
- Temperature of the laboratory thermometer		Y	
- Temperature of the NIST-traceable thermometer (or other reference		· Y	
- Correction (or calibration) factor		Υ	·
- Date of check		Υ	
- Analyst's initials		Υ	
QC Is the thermometer discarded if it differs by more than 1°C from the reference thermometer?	3.3.3	Y	
QC Are reference thermometers recalibrated at least every five years?		Υ	
QC Is reference thermometer calibration documentation maintained?		Y	·
QC Are continuous recording devices used to monitor incubator temperature		<u>.</u>	
recalibrated at least annually, using a reference thermometer that meets the		Y	
specifications noted in Section 3.3.2?	2.4		
Incubator Unit	3.4		<u></u>

		- 1 - 1 / 6	
ELEMENT	MEM	Y/N/O	COMMENTS
Do incubator units have an internal temperature monitoring device and maintain a			
temperature specified by the method used, usually 35°±0.5°C and 44.5°±0.2°C?	3.4.1	Υ	·
	obcooking the same of the same		
For non-portable incubators, are thermometers placed on top and bottom shelves			
of the use area and immersed in liquid as directed by the manufacturer (except		Y	
for electronic thermometers)?	-		
When aluminum block incubators are used, do culture dishes and tubes fit		-	
snugly?		0	
Singly:			
QC Is the calibration-corrected temperature recorded for each thermometer	3.4.2	Y	
being used at least twice per day during each day the incubator is in use?			
QC Are these readings separated by at least four hours?		Υ	
QC Does the documentation include the date and time of reading, temperature,		Υ	
and technician's initials?		1	
If a circulating water bath is used, is it equipped with a gable cover to ensure an		V	
incubation temperature of 44.5E±0.2EC?		Y	
Autoclave	3.5	<b>L</b>	
Does the autoclave have an internal heat source, a temperature gauge with a			
sensor on the exhaust, a pressure gauge, and an operational safety valve?	3.5.1	Y	
sensor on the exhaust, a pressure gauge, and an operational safety valve?	3.3.1	ľ	
Can the autoclave maintain a sterilization temperature during the sterilizing cycle			
and complete an entire cycle within 45 minutes when a 12-15 minute sterilization		Y	
period is used?			
Does the autoclave depressurize slowly enough to ensure that media will not boil		Υ	
over and bubbles will not form in inverted tubes?		Ţ	
QC Is the following information recorded each time the autoclave is used?	3.5.3		
- Date		Υ	
- Contents		Y	
- Sterilization time and temperature	-	Y	
		Y	
- Total time in the autoclave			
- Analyst's initials		Υ	
QC Are copies of the service contracts or internal maintenance protocols and		Y	
maintenance records kept?		<u>'</u>	
QC Is maintenance conducted at least annually?		Υ	
QC Is a record of the most recent service performed on file and available for		Y	
inspection?		T	
QC Is a maximum-temperature-registering thermometer, electronic temperature			
readout device, or continuous recording device used each autoclave cycle to	1	Y	
ensure that the proper temperature was reached?	3.5.4	'	
		Υ	
QC Is the temperature recorded?	1	Y	
QC Is overcrowding avoided?		Y	
QC Are spore strips or spore ampules used monthly as bioindicators to confirm		Y	
sterilization?			
QC Are automatic timing mechanisms checked quarterly with a stopwatch or			
other accurate timepiece or time signal, and the results recorded and initialed?	3.5.5	Υ	
			·
Are autoclave door seals clean and free of caramelized media?	3.5.6	Υ	
Are autoclave drain screens cleaned frequently and debris removed?	3.3.0	Y	
Colony Counter	3.5		·
	3.7		
Is a dark field colony counter used to count Heterotrophic Plate Count colonies?		' Y	
		L	
Conductivity Meter	3.8	:	
Are meters suitable for checking laboratory reagent-grade water and readable in	3.8.1	Y	
units of either micromhos/cm or microsiemens/cm	J.U.1		
QC Is the meter calibrated at least monthly, following the manufacturer's			
recommendations and using an appropriate certified and traceable low-level	3	Y	
standard?			
QC If the meter cannot be calibrated as noted above, is the cell constant		<del> </del>	
	}	^	
determined at monthly intervals using a method in Standard Methods, Section		0	
2510?		ļ	·
Is an in-line unit that cannot be calibrated used to check reagent-grade water?	3.8.3	0	
	2.0.5	U	

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ELEMENT	ITEM	Y/N/O	COMMENTS
Refrigerator	3.9	V	
Does the refrigerator maintain a temperature of 1°-5°C?	3.9.1	Υ	
Is the refrigerator thermometer graduated in at least 1°C increments and the thermometer bulb immersed in liquid?		Y	
QC On days the refrigerator is in use, and the laboratory is staffed, is the	3.9.2	Y	
calibrated-corrected temperature recorded at least once per day?		L	
Inoculating Equipment	3.10	г	
Are sterile metal or disposable plastic loops, wood applicator sticks, sterile swabs, or sterile plastic disposable pipet tips used?		Y	
Are wood applicator sticks, if used, sterilized by dry heat?		0	
Are metal inoculating loops and/or needles made of nickel alloy or platinum?		0	
Culture Dishes (loose or tight lids)	3.12	L	
Are presterilized plastic or sterilizable glass culture dishes used?	3.12.1	Υ	
Is the sterility of the glass culture dishes maintained by placement in stainless		1	
steel or aluminum canisters or a wrap of heavy aluminum foil or char-resistant paper?		Y .	
Are loose-lid petri dishes incubated in a tight-fitting container with a moistened paper towel?	3.12.2	Y	
Are opened packs of disposable culture dishes resealed between use periods?	3.12.3	Υ	
For membrane filter methods, are culture dishes of an appropriate size to allow	3.12.4	0	
the transfer of a single membrane per plate?	3.12.4	U	,
Pipets	3.13	;	·
Are glass pipets sterilized and maintained in stainless steel or aluminum canisters or wrapped individually in char-resistant paper or aluminum foil?	3.13.1	0	
Do pipets have legible markings and are they not chipped or etched?	3.13.2	Υ	
Are opened packs of disposable sterile pipets resealed between use periods?	3.13.3	Y	
Are pipets delivering volumes of 10 mL or less accurate to within a 2.5% tolerance?	3.13.4	Y	
Are calibrated micropipetters used with sterile tips?	3.13.5	Υ	
Are micropipetters calibrated annually and adjusted or replaced if the precision or			
accuracy is greater than 2.5%?		Y	
Glassware and Plasticware	3.14		
Is the glassware made of borosilicate glass, or other corrosion-resistant glass, and free of chips and cracks?	3.14.1	Υ	
Are markings on graduated cylinders and pipets legible?		Υ	·
Are plastic items clear and nontoxic to microorganisms?		Y	-
QC Are the graduated cylinders used for measurement of sample volumes, or	· ·	1	
other precalibrated containers that have clearly marked volumes used in lieu of graduated cylinders, accurate to within a 2.5% tolerance?		Υ.	
Are culture tubes and containers containing fermentation medium of sufficient			
size to contain medium plus sample without being more than three quarters full?	3.14.3	Y	(
Are tube closures made of stainless steel, plastic, aluminum, or screw caps with nontoxic liners?	3.14.4	Υ	
Are cotton or foam plugs used?	1	N	
Sample Containers	3.15		1
Are sample containers wide-mouth plastic or non-corrosive glass bottles with non-			
leaking ground glass stoppers or caps with nontoxic liners, sterile plastic bags containing sodium thiosulfate, or other appropriate sample containers?		Y	
Is sample container capacity at least 120 mL (4 oz) to allow at least a 1-inch head space?		Υ	
Are glass stoppers covered with aluminum foil or char-resistant paper for sterilization?	3.15.2	0	
Are unsterilized glass and plastic bottles sterilized by autoclaving or, alternatively, by dry oven for glass bottles?	3.15.3	0	· .
Are empty containers moistened with several drops of water before autoclaving to prevent an Aair lock@ sterilization failure?		0	
<u> </u>	·		· · · · · · · · · · · · · · · · · · ·

ELEMENT	ITEM	Y/N/O CO	DMMENTS
If chlorinated water is to be analyzed, is sufficient sodium thiosulfate added to the			
sample bottles before sterilization to neutralize any residual chlorine in the water		Y	
sample?			
Ultraviolet Lamp (if used)	3.16		
	3.10		
Is the longwave unit used for fluorometric tests kept clean?		Υ	
4. GENERAL LABORATORY PRACTICES	· · · · · · · · · · · · · · · · · · ·	<del>,</del>	
Are laboratory personnel aware of general and customary safety practices for		Y	
laboratories?			
Does the laboratory have a safety plan available?		Y	
Does the laboratory keep a copy, and follow the personal protection guidelines, of			
any material safety data sheet accompanying the receipt of a toxic material?		Y	
Sterilization Procedures	4.1		
Does the laboratory follow the minimum times for autoclaving the materials listed	J ,		
below at 121°C?	1.1.1		
	<del> </del>	0	<u>.</u>
		0	
- Carbohydrate containing media 12-15 min <sup>1</sup>		Y	
- Contaminated test materials 30 min <sup>2</sup>		Y	
- Membrane filter assemblies 15 min		·Y	
- Sample collection containers 15 min		0	
- Individual glassware 15 min		Y	
- Dilution water blank 15 min		Y	
2	<u> </u>	Y	<del>_</del>
	<del>                                     </del>	' '	
except where otherwise specified by the manufacturer			
<sup>2</sup> time depends upon water volume per container and autoclave load			
Sample Containers	4.2		
QC Is at least one sample container selected at random from each batch of	:		
sterile sample bottles, or other containers (or lot of commercially available sample			
containers), and the sterility confirmed by adding 25 mL of a sterile non-selective		Y	
broth, incubating at 35°±0.5°C, and checking for growth after 24 and 48 hours?			
The same of the sa			
QC Are these results recorded?		Y	
QC If growth is detected, is the entire batch resterilized?		Y	
Reagent-Grade Water	4.2	'	
	4.3		· · · · · · · · · · · · · · · · · · ·
Does the laboratory only use satisfactorily tested reagent water from stills or			*
deionization units to prepare media, reagents, and dilution/rinse water for	4.3.1	Y	
performing microbial analyses?			
- Conductivity >0.5 megohms resistance Monthly*			
or <2 micromhos.cm	4.3.2	Υ	
(microsiemens/cm) at 25°C			•
- Pb, Cd, Cr, Not greater than 0.05 mg/L Annually			
Cu, Ni, Zn per contaminant. Collectively	,	Y	
no greater than 0.1 mg/L			
- Total chlorine <0.1 mg/L Monthly		<del>  </del>	<del></del>
residual*		Y	
- Heterotrophic <500/mL CFU/mL* Monthly		Y	
plate count*			
- Bacteriological Ratio of growth rate Annually			
quality of 0.8 to 3.0		0	
reagent water*		<u>                                     </u>	
*See Section 4.3.2 for footnotes			
Dilution/Rinse Water	4.4		
Is stock buffer solution or peptone water prepared as specified in Standard	ıl		
Methods, Section 9050C?	4.4.1	Υ	
Are stock buffers autoclaved or filter-sterilized?	4.4.2	Y	
	7.4.2	Y	
Are these containers labeled, dated, and refrigerated?		<del></del>	
Are stored stock buffers free from turbidity?	<u> </u>	Y	
QC Is each batch (or lot, if commercially prepared) of dilution/rinse water			•
checked for sterility by adding 50 mL of water to 50 mL double strength non-		Y	•
selective broth, incubating at 35°± 0.5°C, and checking for growth after 24 hours		'	
and 48 hours?			

ELEMENT	ITEM	V/N/O	COMMENTS
QC Are these results recorded?	LI ENI	Y	COMMENTS
QC Is the batch/lot discarded if growth is detected?		Y	
Glassware Washing	4.5		
Is distilled or deionized water used for the final rinse?	4.5.1	Υ	
Is laboratory glassware washed with a detergent designed for laboratory use?	4.5.2	Υ	
QC Is the glassware inhibitory residue test performed before the initial use of a washing compound and whenever a different formulation, or washing procedure is used?		Y	
QC Are these results recorded?		Y	
QC Is each batch of dry glassware used for microbial analysis spot-checked for pH reaction using 0.04% bromthymol blue (or equivalent pH indicator) and the color reaction recorded?		Y	
5. ANALYTICAL METHODOLOGY			
General	5.1		
For compliance samples, does the laboratory use only the analytical methodologies specified in the Total Coliform Rule (TCR), the Surface Water Treatment Rule (SWTR), and the Groundwater Rule (GWR)?	5.1.5	Y	·
Is the laboratory certified for all analytical methods it uses for compliance purposes?	5.1.2	Y	
At a minimum, is the laboratory certified for one total coliform method and one		Υ	
fecal coliform or E. coli method?  For a laboratory that enumerates heterotrophic bacteria for compliance with the SWTR, is the laboratory certified for either the Pour Plate Method or the SimPlate		Υ	
method for heterotrophic bacteria?			
Are water samples shaken vigorously at least 25 times before analyzing?	5.1.3	Y	
QC If dilution buffer is used, does the laboratory check the buffer volume in one dilution bottle of each batch or lot?	5.1.4	0	
QC For a 90-mL or 99-mL volume, is the tolerance ±2 mL?		1.	
		0	SAMPLE VOLUME PIPETED AND BROUGHT TO 100 ML WITH IDEXX BOTTLE 100 ML MARK
Does the laboratory analyze a 100-mL sample volume for total coliforms in drinking water?	5.1.5	Y	
Media (or defined substrate)	5.1.6		
Are dehydrated media stored in a cool dry location and discarded by the manufacturer's expiration date?	5.1.6.1	Y	
Is caked or discolored dehydrated media discarded?		Y	
QC For media prepared in the laboratory is the following information recorded?	5.1.6.2	Υ	
- Date of preparation		Y	
- Type of medium		Ÿ	
- Lot number		Ý	
- Sterilization time and temperature		Ý	
- Final pH (after sterilization)		Ÿ	
- Technician's initials		Y	
QC For media prepared commercially is the following recorded for each lot?	5.1.6.3		
- Date received		Y	·
- Type of medium		Y	
- Lot number		Υ	
- pH verification		Y	·
QC Are media prepared commercially discarded by manufacturer's expiration date?		Y	
QC Is each new lot of dehydrated or prepared commercial medium and each batch of laboratory-prepared medium checked before use for sterility and with positive and negative culture controls?		Y	
QC Are these results recorded?		Y	·
QC For laboratories using commercially prepared media with manufacturer shelf lives of greater than 90 days, are positive and negative controls run each quarter, in addition to that noted above?		Y	
			·

ELEMENT	ITEM	V/N/O	COMMENTS
	ITEM	YNVO Y	COMPLENTS
QC Are these results recorded?	-	T	· · · · · · · · · · · · · · · · · · ·
QC For control organisms, are stock cultures periodically checked for purity and			
the results recorded, or are commercially available disks impregnated with the		Υ	;
organism used?			
If prepared medium is stored after sterilization, is it maintained in the dark as	5.1.6.5	V	
follows?	5.1.6.5	Y	
- poured plates 1° - 5°C 2 weeks	· .	Y	
- broth in containers with 1° - 30°C 2 weeks	<del>                                     </del>		
· ·		Υ	
loose-fitting closures	<u> </u>		
- broth in tightly closed 1° - 30°C 3 months		Υ	
containers			
QC Does the laboratory perform parallel testing between a newly approved test			
and another EPA-approved procedure for enumerating total coliforms for at least	.}		
several months and/or several seasons to assess the effectiveness of the new		Υ	
test for the wide variety of water types submitted for analysis? Recommended.			
Took for the vide failety of videor types dublinited for allaryole. The commended.			,
Does the laboratory perform the approved methods listed in this section for the	5.1.8	Υ	•
TCR, SWTR, and/or GWR?		•	
Enzyme (chromogenic/fluorogenic) substrate tests	5.3		
General	5.3.1		
For detecting total coliforms and E. coli in drinking water by an enzyme substrate			
test, does the laboratory use one of the following: MMO-MUG test (Colilert),			`
Colisure test, E*Colite test, Readycult Coliforms 100 Presence/Absence Test,		Υ	_
Fluorocult LMX test, or Colitag test?			,
For enumerating total coliforms in source waters by an enzyme substrate test,		Υ	
does the laboratory use the Colilert test?		'	·
If a laboratory uses a fermentation method to detect total coliforms in drinking			
water, and the sample is total coliform-positive, does the laboratory transfer the			
positive culture to the EC+MUG test to detect E. coli, but not to any other enzyme		Υ	
substrate test medium in Section 5.3?			
Media	5.3.1.2		-
Does the laboratory purchase media from a commercially available source only,	5.3.1.2.1	Υ	
and not prepare media from basic ingredients?	3.3.1.2.1	Ť	•
Are media kept protected from light?	5.3.1.2.2	Υ	
Is each lot of medium checked for fluorescence before use with a 365-366-nm	-		
ultraviolet light with a six watt bulb?	5.3.1.2.3	Υ	,
<u> </u>			
If medium exhibits faint fluorescence, is another lot used that does not fluoresce?	1	Ϋ́	
If samples plus medium exhibit color changes before incubation, is the medium	5.3.1.2.4	V	
discarded and another lot of medium used?	5.3.1.2.4	Y	
Are glass and plastic bottles and test tubes checked before use with a 365-366-	1		
nm ultraviolet light source with a 6-watt bulb to ensure that they do not fluoresce?		Y	
I thin diliaviolet light source with a o-walt build to ensure that they do not hidoresce:	3.3.1.3	T	
		· · ·	
If they fluoresce, does the laboratory use another lot of containers that does not		Y	
fluoresce?		•	·
If a Whirl-Pak7 bag is used to incubate the Colilert or Colitag medium or any other			
medium which changes to a yellow color to indicate a positive result, is a type		_	
used that has a barrier (e.g., B01417) to prevent gaseous emissions to other		0	
Whirl-Pak7 bags during incubation?			
QC If a small air-type incubator is used, are samples brought to room	5.3.1.5	0	
temperature before incubation?	5.5.1.5		
If a water bath is used, is the water level above the upper level of the medium?	5216		
\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	5.3.1.6	Υ	
For E. coli testing, are all total coliform-positive samples placed under a UV lamp	-		
	5.3.1.7		
(365-366 nm) in a darkened area?			
Does the laboratory refrain from using the enzyme substrate test to confirm a			
presumptive total coliform-positive culture in a fermentation broth or on a	5.3.1.8	Υ	
membrane filter?		'	

	VOCAL	1:01/0	CONNENTS
ELEMENT	ITEM	Y/N/O	COMMENTS
Does the laboratory invalidate any sample that produces an atypical color change			
(in the absence of a yellow color) and then collect, or request that the system	5.3.1.9	Υ	
collect, another sample from the same location as the original invalidated	3.3.1.9	'	
sample?			
Does the laboratory use another method to test the second sample?		N	
Is the reference comparator provided by the manufacturer discarded by the			
manufacturer's expiration date?	5.3.1.10	Υ	
	5.3.2		
Criteria for specific media		\ <u>'</u>	
For the Colilert test, are samples incubated at 35°±0.5°C for 24 hours?	5.3.2.1	Υ	
Is a sample with a yellow color in the medium equal to or greater than reference		Υ	
comparator recorded as total coliform-positive?			
Is a sample with a yellow color lighter than comparator incubated for another four		Υ	
hours but no longer than 28 hours total?		,	
Is a sample with a yellow color lighter than the comparator after 28 hours of		Y	
incubation recorded as total coliform-negative?		Τ .	
Are coliform-positive samples that fluoresce under a UV light marked as E. coli-	·		
positive?		Υ	
For the Colilert-18 test, are samples incubated for 18 hours (up to 22 hours if the			
sample after 18 hours is yellow, but lighter than the comparator)?		Υ	
For enumerating total coliforms in source waters, does the laboratory use the		v	
Colilert test, a 5- or 10-tube configuration, Quanti-Tray, or Quanti-Tray 2000 for	5.3.2.1.1	Υ	
each sample dilution tested?			
When dilution water is used, is it either sterile deionized or sterile distilled water,		Υ	
not buffered water?		T	
QC If the Quanti-Tray or Quanti-Tray 2000 test is used, is the sealer checked			
monthly by adding a dye to the water?	5.3.2.1.2	Y	NEEDS DONE JAN 2012
For the Colisure test, are samples incubated at 35°±0.5°C for 24-48 hours?	5.3.2.2	0	
The state of the s			
If the medium changes from a yellow color to a red/magenta color, is the sample		0	
noted as total coliform-positive?			
Is a coliform-positive sample that fluoresces under a UV light marked as E. coli-		0	
positive?			·
For the E*Colite test, is the sample incubated at 35°±0.5°C for 28 hours?	5.3.2.3	0	,
If the medium changes from a yellow color to a blue or blue-green color, or a blue			
color in the corners of the bag, is the sample marked as total coliform-positive?		0	
If the medium fluoresces under a UV light, is the sample considered as E. coli-			
positive?		0	
If fluorescence is not observed, is the sample reincubated for an additional 20			
		•	
hours (for a total incubation time of 48 hours) and checked again for	*	· 0	·
fluorescence?			
If the medium becomes red in color, is the sample discarded and another sample		0	•
requested?			
For the Readycult Coliforms 100 Presence-Absence test, are the contents of a			·
snap pack added to a 100-mL sample and then incubated at 35°±0.5°C for 24±1	5.3.2.4	. 0	
hours?			·
If the medium changes color from a slightly yellow color to blue-green, is the			
sample marked as coliform-positive?		0	
If the medium fluoresces a bright light-blue color when subjected to long wave UV			
		0	
(365-366 nm) light, is the sample marked as E. coli-positive?			
For the Fluorocult LMX test, is the medium added to purified water, mixed, and	5.3.2.5	0	
the mixture then boiled to dissolve the medium completely in the water?			
Are 100-mL aliquots transferred to 250-mL bottles and then autoclaved for 15		0	
minutes?			
Are the autoclaved bottles cooled before adding the 100-mL water sample?		0	
Is the E. coli/Coliform Supplement not added to the medium?		0	
Is the sample then incubated at 35°±0.5°C for 24±1 hours?		0	
If the medium changes color from a slightly yellow color to blue-green, is the			
1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7		0	· 
sample marked as coliform-positive?			
If the medium fluoresces a bright light-blue color when subjected to long wave UV		0	
(365-366 nm) light, is the sample marked as E. coli-positive?			
For the Colitag test, are samples incubated at 35°±0.5°C for 24±2 hours?	5.3.2.6	Υ	

ELEMENT	ITEM	V/N/O	COMMENTS
If the medium changes to a yellow color, is the sample marked as coliform-		Y	COMMENTS
positive?		Y	
If the medium fluoresces under a UV light, is the sample marked as E. coli-		Υ	
positive?			
EC Medium + MUG (for detection of E. coli)	5.3.3	0	
If EC medium + MUG is used, is a total coliform-positive culture transferred from a presumptive tube/bottle or colony to this medium?	5.3.3.1	0	• .
Is the final pH of EC medium + MUG 6.9±0.2?	5.3.3.2	0	<u> </u>
Is the medium plus sample incubated at 44.5°±0.2°C for 24±2 hours and then		U	
tested for fluorescence?	5.3.3.4	0	
Enterolert test (for detection of enterococci in ground water)	5.3.4	0	
Is the medium stored in the dark at 4°-30°C until used?	5.3.4.1	0	
Is Enterolert reagent added to a 100-mL sample and the sample/medium		_	
incubated at 41°±0.5°C for 24-28 hours?	5.3.4.2	0	
Is fluorescence under a UV lamp used to indicate the presence of enterococci?		0	
Heterotrophic Plate Count (for enumerating heterotrophic bacteria in			
drinking water)	5.5		
Does the laboratory use the Pour Plate Method or the SimPlate Method for			
enumerating heterotrophic bacteria in drinking water and for testing reagent grade	5.5.1	Υ	
water?			
For systems granted a variance from the TCR's maximum contaminant level,			
does the laboratory use R2A medium with a method in Standard Methods,		0	,
Section 9215 for enumerating heterotrophic bacteria in drinking water?			
Media	5.5.2		
Is the final pH recorded for plate count agar pH 7.0±0.2, R2A agar 7.2±0.2, and			
SimPlate 7 2+0 22		Υ	
For the Pour Plate Method, is melted agar tempered at 44°-46°C in a water bath	553	Υ	•
and maintained no more than 3 nours before pouring?	3.3.3		
Is this sterile medium melted only once?		Y	
For the Spread Plate Method, is 15 mL of R2A medium (or other medium) poured	5.5.4	0	
into a sterile petri dish and allowed to solidity?			
Is refrigerated medium in bottles or screw-capped tubes stored for no longer than six months, or in petri dishes for no longer than 2 weeks (one week for prepared		Υ	·
netri dishes with R2A medium)?		1	
For countable plates of most potable water samples, are 1.0 mL and/or 0.1 mL			
volumes of the undiluted sample plated?	5.5.6	Υ.	·
Are at least duplicate plates prepared per dilution tested?		Υ	
For the Pour Plate Method, is the sample pipetted aseptically onto the bottom of a			
sterile petri dish and then at least 10-12 mL tempered melted agar added?	5.5.7	Y	
Is the sample and melted agar mixed, avoiding spillage?		Υ	
After the agar plates have solidified on a level surface, are they inverted and		Υ	
incubated at 35°±0.5°C for 48±3 hours?			
Are plates stacked no more than four high and arranged in the incubator to allow proper air circulation and to maintain a uniform incubation temperature?		Y	
proper all circulation and to maintain a uniform incubation temperature?		T	
Does the laboratory ensure that incubator does not have excess humidity and that			
the plates do not lose more than 15% by weight during the 48 hours of		Υ	
incubation?		,	
For the Spread Plate Method, is 0.1 or 0.5 mL of the sample (or dilution) pipetted			
onto the surface of the predried agar in the plate and then spread over the entire		0	
surface using a sterile bent glass rod?			
Is the inoculum absorbed completely before incubating?		0	
Are the plates incubated in an inverted position at 20°-28°C for 5-7 days?		0	
For the Membrane Filter Technique, does the filtered volume yield between 20-200 colonies?	5.5.9	0	,
Is the filter transferred to a petri dish containing 5 mL solidified R2A medium and			
then incubated at 20°-28°C for 5-7 days?		0	
	1		· · · · · · · · · · · · · · · · · · ·

FLEMENT	(TEA)	VINIO	COMMENTS		•
Are plates with loose-fitting lids placed in a plastic box with a close-fitting lid and	ITEM	Y/N/O	COMMENTS		
moistened paper towels, and rewetted as necessary?	•	0			
Are colonies counted using a stereoscopic microscope at 10-15X magnification?	-	-			
The deletined delined delined a stored deline interesting at 10 Text magnification.		0			
SimPlate Method	5.5.10	0		<del>`</del>	-
For a single sample Unit Dose, is a 10-mL test sample added to a test tube	<del></del>	1		<del>.</del>	
containing dehydrated SimPlate medium and then poured onto the center of a		0			
plate containing 84 small wells?					
Alternatively, is 9-mL of sterile diluent added to the test tube containing the					
dehydrated medium, followed by a 1-mL sample, and the medium plus sample		0			
then poured onto the center of a plate containing 84 small wells?		-			
Is this mixture distributed evenly to the 84 wells and is the excess liquid drained		0			
into the absorbent pad on the plate?			1		
Is the plate inverted and incubated at 35°±0.5°C for 45-72 hours?		0			
Is bacterial density determined by counting the number of wells that fluoresce					
under a 365-366-nm UV light, and converting this value to a Most Probable		0			
Number/mL using the manufacturer's Unit Dose MPN table?					
If a 10-mL sample is used, is the Unit Dose MPN/mL read directly or, if a 1-mL	-	0			
sample is used, is the MPN/mL value corrected by multiplying it by 10?		<u></u>			
For the Multiple Dose for 10 samples of 1 mL each, is a 100-mL sterile diluent	5 5 10 2	0	-		
added to the denydrated SimPlate medium and snaken to dissolve?					•
Is a 1.0-mL test sample then pipetted to the center of a plate, followed by 9 mL or	f	0			
the reconstituted medium?		ļ · °			
Is the plate then gently swirled to mix and distribute the sample and medium	3				
mixture evenly to the 84 wells, with the excess liquid then being drained into the	<b>:</b>	0			
absorbent pad on the plate?					
Is the plate inverted and incubated at 35°±0.5°C for 45-72 hours?		0			
Is bacterial density determined by counting the number of wells that fluoresce		'			
under a 365-366-nm UV light, and converting this value to a Most Probable		0			
Number/mL using the manufacturer's Multi-Dose MPN table?					
If sample dilutions were made during sample preparation, is the MPN/mL value		0			
multiplied by the dilution factor?					
For the Pour Plate and Spread Plate Techniques, are colonies counted manually	5.5.11	Y			
using a dark field colony counter?		-			
Are only plates having 30 to 300 colonies counted, except for plates inoculated		١,,			
with 1.0 mL of undiluted sample where counts of less than 30 are acceptable?		Y			
QC Is each batch or flask of agar checked for sterility by pouring a final control	5.5.12	Υ			,
plate?	-	Υ			<del></del>
QC Does the laboratory reject data if the control is contaminated?  6. SAMPLE COLLECTION, HANDLING, AND PRESERVATION	<u> </u>	T	<u> </u>		·
Sample Collector	6.1	_			
Is the sample collector trained in aseptic sampling procedures and, if required		-	T.		
approved by the appropriate regulatory authority or its designated representative?		Y	•		
approved by the appropriate regulatory authority of its designated representative:		1			
Sampling	6.2				
Are the drinking water samples collected under the Total Coliform Rule			Ī		
representative of the water distribution system?	6.2.1	Y			
Are the water taps used for sampling free of aerators, strainers, hose	-	+		_ <del>.</del>	
attachments, mixing type faucets, and purification devices?		Y			
Are only cold water taps used?		Y			
Are service lines cleared before sampling by maintaining a steady water flow for	-				
at least 2 minutes or until a steady water temperature is reached?		Y			
Is at least a 100-mL sample volume collected, allowing at least a 1-inch air space	`	<u> </u>			
in the container to facilitate mixing of the sample by shaking?		Y			
Is a sample information form completed immediately after sample collection?		<u> </u>	1.		
The state of the s		Y			
If a sample bottle is filled too full to allow for proper mixing, is the entire sample					
poured into a larger sterile container and mixed before proceeding with the		Υ		•	
analysis?					

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ELEMENT	ITEM	VINIO	COMMENTS
For the SWTR, are the source water samples representative of the source of	ITEM	1/N/O	COMMENTS
supply and collected not too far from the intake point, but at a reasonable		Υ	
distance from the bank or shore?	,	•	
Is the sample volume sufficient to perform all the tests required?		Υ	
For the analysis of coliphage, E. coli, or enterococci under the GWR, is at least a			
100-mL sample volume collected?	6.2.3 6.2.4	Υ	
Sample Icing	<b>6.3</b> .		
For drinking water bacterial samples, is the sampler encouraged to hold samples	1	,	
at <10°C during transit to the laboratory?	6.3.1	Υ	
For source water bacterial samples, are samples held at <10°C during transit to			
the laboratory?		Υ	
Does the laboratory reject samples that have been frozen?		Y	
For coliphage analysis under the GWR, are samples shipped at <10°C, stored at		<u>'</u>	-
1°-5°C, and not frozen?	6.3.2	0	·
QC For SWTR samples and coliphage samples, does the laboratory record			
sample temperature upon receipt?		0	
QC Does the laboratory flag samples that have a temperature upon receipt of			
>10°C, whether iced or not, unless the time since the sample collection is less		.Υ	
than two hours?		. •	
Sample Holding/Travel Time	<b>6.4</b> .		
For the analysis of total coliforms in drinking water, does the time between		_	
sample collection and placement of the sample in the incubator not exceed 30		Υ	
hours?	0.1.1	•	
Are all samples analyzed on the day of receipt?		Y	
Are samples received late in the day refrigerated overnight only if analysis can			
begin within 30 hours of collection?		Y	
For total coliforms and fecal coliforms in surface water sources, and for			
heterotrophic bacteria in drinking water, is the time from sample collection to	1	Υ	
placement in the incubator less than eight hours?	0.1.2	•	
For E. coli and enterococci analysis under the GWR, is the time between sample			
collection and the placement of sample in the incubator less than 30 hours?	6.4.4	Υ	
benedich and the placement of cample in the incastion loss than so hears.	0.7.7	•	
Sample Information Form	6.5		
After collection, does the sampler enter the following information, in indelible ink,			
on sample information form?			
- Name of system (PWSS identification number if available)		Y	
- Sample identification (if any)		Y	
- Sample site location		Y	
- Sample type (e.g., a routine distribution, repeat, raw or process, or			
other special purpose)		Y	
- Date and time of collection		Y	
- Analysis requested		Y	
- Disinfectant residual		Y	
- Name of sampler		Υ	
Any remarks		Y	
Chain-of-Custody	6.6	,	
Are applicable State regulations pertaining to chain-of-custody followed by			
sample collectors and the laboratory?		Y	
7. QUALITY ASSURANCE	l		
Does the laboratory have a written QA Plan prepared and available for			<u> </u>
inspection?	7.1	Υ	
Does the laboratory follow the written QA Plan?		Υ	
Does the laboratory have a Standard Operating Procedure available for review			
pertaining to its own calibration of equipment or supplies?		Υ	·
Door the laboratory eugeneratilly engine at least one act of DT complete	· .	L	
Does the laboratory successfully analyze at least one set of PT samples	7.2	Υ	
once every 12 months for each method for which it is certified?	I		
For methods used to test the presence or absence of an organism in a sample, does the laboratory analyze each PT sample set using a single analytical method			
does the laboratory analyze each PT sample set using a single analytical method		Y	P. C. T. 性質的 用 (1) (1) (1) (1) (1)
only?	L	L	
8. RECORDS AND DATA REPORTING Legal Defensibility	0.1		·
	8.1		

ELEMENT	ITEM	Y/N/O	COMMENTS		
Are compliance monitoring data being maintained by the laboratory both thorough		1			
and accurate, and thus legally defensible?		Y			
Does the laboratory's QA plan and/or SOPs describe the policies and procedures	3	1			
used by the facility for record retention and storage?		Y			
If samples are expected to become part of legal action, does the laboratory follow	,				
chain-of-custody procedures?		Υ			
Maintenance of Records	8.2				
Does the public water system maintain records of microbiological analyses for five		Y		,	
years?					
Does the laboratory maintain easily accessible records for five years or until the	9	Y			
next certification data audit is completed, whichever is longer?		1.			
Does the laboratory notify the client water system before disposing of records so	) ··	Y			
they may request copies if needed?		<u>'</u>			
Does the laboratory backup all electronic data by protected tape, disk, or hard	1	Y			
copy?					
When the laboratory changes its computer hardware or software, are provisions	3				
in place for transferring old data to the new system so that data remain retrievable		Y		•	
within the specified time frames?		'			
Sampling Records	8.3				
Are all data recorded in ink, with any changes lined through such that the original	'	Y			
entry is visible?		1			
Are changes initialed and dated?		Y			
Does the laboratory have the following sample information readily available?	8.3.1-4				
- Date and time of sample receipt by the laboratory		Υ			
- Name of the laboratory person receiving the sample		Y			
- Information on any deficiency in the condition of the sample		Y			
Are samples invalidated for the following reasons?	8.3.4	'	·		
	0.3.4	V			
- Time between sample collection and receipt by laboratory exceeded		. Y	•		
- Presence of disinfectant in sample noticed, e.g., odor		Y			
- Evidence of freezing		Y			
<ul> <li>Use of a container not approved by the laboratory for the purpose</li> </ul>		Y			
intended		'			
- Insufficient sample volume, e.g., <100 mL		Y			
- Presence of interfering contaminants noticed, e.g., hydrocarbons,		1			
cleansers, heavy metals, etc.		Y			
- Sample temperature exceeding the maximum allowable	+	Y			
Analytical Records	0.4				
, and y area. There is a	8.4				
Are all recorded data in ink with any changes lined through such that origina	I	Y			
entry is visible?					
Are these changes initialed and dated?		Υ			
Are the following readily available?	8.4.1-6				
- Laboratory sample identification information		Υ			
- Information concerning date and time analysis begins		Υ			
- Name of the laboratory and a signature or initials of the person(s)					
performing analysis		Υ			
- Information concerning the analytical technique or method used	-	Y			
- Information concerning all items marked "QC"	ļ	Y	•		
- Results of the analyses	K.	Υ			
Preventive Maintenance	8.5				
Does the laboratory maintain preventive maintenance and repair records for al	t	Y			
instruments and equipment?		'			
Are these records kept for five years in a manner that allows for easy inspection?					
,		Y			
9. ACTION RESPONSE TO LABORATORY RESULTS		<u> </u>	<del> </del>		
	0.1				
Testing Total Coliform-Positive Cultures	9.1				
For the Total Coliform Rule, does the laboratory test all total coliform-positive	*	Y			
cultures for the presence of either fecal coliforms or E. coli?					
Notification of Positive Results	9.2				
For Total Coliform Rule, does the laboratory promptly notify the proper authority of	f				
		1 v			
a positive total coliform, fecal coliform, or E. coli result, so that appropriate follow-	-   9.2.1	Y			

ELEMENT	ІТЕМ	Y/N/O COMMENTS
For the Total Coliform Rule, if a sample is fecal coliform- or E. coli-positive, does the system notify the State as soon as it is notified of the test result, i.e., at the end of that day or, if the State office is closed, by the end of the next business day?	0.2.2	Υ
Does the laboratory base a total coliform-positive result on the confirmed phase if the Multiple Tube Fermentation Technique or Presence-Absence Coliform Test is used, or the verified test for the Membrane Filtration Technique if M-Endo medium or M-Endo LES agar is used?	0.2.2	Y
If a presumptive total coliform-positive culture does not confirm/verify as such, but is found to be fecal coliform or E. coli-positive, is the sample considered total coliform-positive and fecal coliform/E. coli-positive?		Y
Notification of Total Coliform Interference	9.3	
For the Total Coliform Rule, does the laboratory promptly notify the proper authority when results indicate non-coliforms may have interfered with total coliform analysis?		Y
TOTAL ITEMS REVIEWED:	274	
NUMBER OF ITEMS MEETING THE MINIMUM REQUIREMENTS:	272	
NUMBER OF ITEMS NOT IN COMPLIANCE WITH MINIMUM REQUIREMENTS:		
LABORATORY SCORE	99%	